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TOLERANCE FOR ALKALIES IN ASIATIC CHOLERA.[1]

By ANDREW WATSON SELLARDS.

(*From the Biological Laboratory, Bureau of Science.*)

The symptoms of Asiatic cholera, in comparison with those of other acute bacterial infections, present some unusual features. Although the infectious process is extremely acute, the disease sometimes running a fatal course in a few hours, yet the body temperature is usually normal or subnormal during the stage of collapse. In this stage, a pronounced disproportion frequently occurs between the temperature and the pulse rate; for example, a temperature of 36° or 37° C. may be accompanied by a pulse rate varying perhaps from 130 to 160 per minute. In contrast to the other infections of the intestine such as tuberculosis, typhoid fever, and bacillary dysentery, there is a marked leucocytosis comparable only to that which is sometimes seen in pneumonia.

Certain features of the disease, such as the short course, the abrupt onset, and the low body temperature, bear more resemblance to the acute intoxications of chemical origin than to the ordinary infectious fevers. During the reaction from the state of collapse the symptoms of toxæmia develop, but the origin of the toxin is not entirely clear. In addition to the hypothetical toxin of the cholera vibrio, the sudden loss of large quantities of fluid[2] from the body gives rise to a considerable mechanical disturbance in the circulation of the blood; it has been suggested that this disturbance might be responsible for all of the symptoms of cholera. However, there are fulminating cases of cholera "sicca," where the loss of fluid is negligible; also, the early relief or prevention of the mechanical disturbance by the administration of fluid to the body, often fails to control the disease.

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[1] Presented at the first biennial meeting of the Far Eastern Association of Tropical Medicine, held at Manila, March 8, 1910.

The principal evidence indicating serious metabolic disorder is the rather high percentage of cases which die in uræmia, amounting in some epidemics to 20 per cent (2). Consequently, the treatment of this complication becomes second in importance only to the treatment of collapse. The development of nephritis and uræmia may be dependent both on the toxæmia and on the mechanical disturbance. Mild cases of cholera, with insignificant loss of fluid, not infrequently develop a fatal uræmia. Also, Czerny (3) has shown that when laboratory animals are rapidly deprived of water, recovery takes place on the restoration of fluid, but a temporary albuminuria develops. The following investigation will consider the possible relation between uræmia and certain symptoms of acid intoxication occurring in Asiatic cholera.

The material on which the following data are based consisted of a series of cholera cases occurring almost exclusively among the Malay races in Manila from September, 1909, to January, 1910, inclusive. The total number of cases was distributed with fair uniformity throughout this period. The disease was widely scattered over the city and outlying districts. There was apparently no direct communication between the individual foci of infection, neither was any common source of infection located. The outbreak was rather more endemic than epidemic in character. Of the total of 340 cases, 147 were found dead by the municipal medical inspectors and the remaining 193 were required by the regulations of the Bureau of Health to be transferred to the San Lazaro Hospital. Bacteriologic examination of all cases for the isolation of the cholera vibrio in pure culture was performed by the biological laboratory of the Bureau of Science. Only the cases where the injection of salt solution was indicated, 111 in number, were studied in this investigation. It gives me much pleasure to acknowledge the many courtesies of Dr. A. P. Goff, chief of the San Lazaro Hospital Division of the Bureau of Health.

[2] The loss of fluid is measurable in liters. The desiccation of the tissues is very general, but the effect on the blood is most noticeable. Rogers (1) found that, instead of the corpuscles forming about 45 per cent of the total volume of the blood, their volume may rise to as much as 60 to 80 per cent, and the fluid content of the blood may be reduced as much as 64 per cent of the total. The viscocity of the blood becomes so great that circulation through the capillaries is effected only with the greatest difficulty. This increased viscocity is probably sufficient to account for the rapid pulse rate, even in the absence of fever. With the continued loss of fluid, the blood pressure in the brachials very commonly falls to zero and the peripheral arteries may remain pulseless for many hours.

In looking for evidences of acid intoxication attention was directed especially to the examination of the urine[3] in regard to: (1) Acetone and acetoacetic acid; (2) the ammonia coefficient; (3) tolerance of the body to alkalies, *i. e.*, the amount of alkali required by the body to render the urine alkaline.

Acetone and acetoacetic acid.—The urine was examined directly, without distillation, for acetone using the iodoform test and the nitro-prussid color reaction. Twenty individuals receiving treatment with sodium chloride were examined. Specimens were obtained from both mild and severe cases including urines voided in collapse, in uræmia and in convalescence. The reactions for acetoacetic acid were somewhat inconclusive. With ferric chloride a definite Bordeaux red color was not obtained, but in seven of the twenty cases a dark red and, in some instances, a slightly brownish-red color was obtained. It is not unlikely that the urinary pigments may have masked the violet shades which are obtained with acetoacetic acid in aqueous solution. Furthermore, the red color faded to a yellow on standing after a few hours; also when the urine was first boiled, no color reaction was obtained. However, one would hesitate to conclude definitely that acetoacetic acid was present, especially as none of the specimens gave a definite reaction for acetone. The absence of acetone seems a little unexpected when we consider that in cholera at least three conditions are present which sometimes give rise to acetonuria, namely, starvation, intestinal disorder, and uræmia.

According to von Noorden (13) acetone and acetoacetic acid usually appear in the urine on the first day of starvation in healthy individuals. Ewing (14) has collected many instances of acetonuria in intestinal disturbances which have been characterized especially as cyclic vomiting and coma dyspepticum. In two cases of uræmia, Lorenz (15) found acetone, acetoacetic acid and β oxybutyric acid.

More extensive observations might offer some explanation for the failure of acetone to appear in the urine; perhaps a larger variety of specimens from different stages of cholera might show that it is not constantly absent.

Ammonia coefficient.—Of course, the ammonia excreted in the urine gives some information as to the amount of acids produced in the body. V. Terray, Vas, and Gara (6) found a pronounced increase in the excretion of ammonia during the stage of reaction and this increase was both relative and absolute, the highest amount recorded being 6.5 grams in one day. In the examination of 28 cases we have found, almost uniformly, an increase in the ammonia coefficient. There were but 6 cases which at any time gave a coefficient of less than 5 per cent, and 3 of these were receiving alkali or acetate in relatively large amounts. In some instances the increase was comparable only to the values obtained in the acid intoxication occurring in diabetes. The data are given in Table VIII.

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[3] No examinations of the blood were attempted. Clinical determinations of the carbon dioxide content of the blood are as yet unsatisfactory. Apparently the normal alkalinity of the blood in cholera is definitely reduced (4). Cantani (5) has even reported an acid reaction of the blood at the end of the life.

Tolerance for alkalies.—If, in cholera, there is an increased production of ammonia for the neutralization of acids, then the administration of alkalies might result, first, in a reduction of the ammonia coefficient, or, second, the amount of alkali which is normally required to render the urine alkaline might be increased.

Preliminary experiments testing the ingestion of sodium bicarbonate by mouth suggested a fairly definite increase in tolerance. The principal source of error attending ingestion experiments would lie in the possibility of a loss of alkali before assimilation. With the expectation of at least partially avoiding such an error, the alkali was given in relatively small quantities and repeated at frequent intervals until the urine became alkaline. Patients were selected who were not in a stage of active diarrhœa; also, only those cases were chosen which were voiding rather freely in order that the results might not be complicated by any marked disturbances in the secretion of urine. Because of these restrictions none of the cases selected showed clinical signs suggesting acid intoxication. In Table I those cases are excluded in which any marked diarrhœa followed the use of the sodium bicarbonate.

TABLE I. –*Showing effect of ingestion of alkalies.*

ONE GRAM SODIUM BICARBONATE EVERY TWO HOURS.

Serial number.	Day of disease.	Condition of patient.	Number of stools.	Total amount of alkali.	Effect on reaction of urine to litmus.	Termination.
				Grams.		
1	22	Recovered. One week without symptoms.	0	7	Alkaline for 2 hours.	
2	15	Convalescent (3 days).	0	7	All specimens acid	Recovery.
3	13	Convalescent (1 week).	0	[a]4	do	Do.
4	5	Fair condition	1	7	do	Do.
5	3	Partial collapse	2	7	do	Do.
Control			0	2	Neutral or alkaline for 14 hours.	

TABLE I.—*Showing effect of ingestion of alkalies*—Continued.

FOUR GRAMS SODIUM BICARBONATE EVERY TWO HOURS.

Serial numbers.	Day of disease.	Condition of patient.	Number of stools.	Total amount of alkali.	Effect on reaction of urine to litmus.	Termination.
				Grams.		
7	3	Mild symptoms	0	20	All specimens acid	Recovery.
5	4	In stage of reaction	1	12	Neutral for 2 hours	Do.
6	8	Early uræmia	7	20	All specimens acid	Death.
4	6	In stage of reaction	1	20	do	Recovery.
10	?[a]	Early uræmia	3	28	do	Death.

EIGHT GRAMS SODIUM BICARBONATE EVERY TWO HOURS.

Serial numbers.	Day of disease.	Condition of patient.	Number of stools.	Total amount of alkali.	Effect on reaction of urine to litmus.	Termination.
7	4	Mild symptoms	1	16	Alkaline	Recovery.
8	3	Early uræmia	2	32	All specimens acid	Death.
Normal			0	8	Neutral or alkaline for 7 hours.	
Do			0	8	Neutral or alkaline for 6 hours.	

[a] Bicarbonate discontinued on account of nausea.

In order to eliminate some of the sources of error arising from the ingestion of alkalies, intravenous injection was also tested. In Table I, one patient (Number 8), in addition to the alkali by mouth, received intravenously 40 grams of sodium bicarbonate followed twelve hours later by 10 grams of sodium carbonate, also injected intravenously; the urine remained acid. Intravenous injections of sodium bicarbonate were then taken up systematically.

The majority of the patients were first tested immediately on admission, while in collapse. As a routine, the bladder was emptied by catheter before each injection of alkali. Because of the anuria of cholera there was, in some instances, an unavoidably long interval between the injection of alkali and the first secretion of urine; therefore, these intervals have been included with the other data. The amounts of sodium bicarbonate are stated in terms of the original amount present before sterilization. It is important to note that after sterilization in the autoclave, approximately 0.2 per cent of normal sodium carbonate was present in the solution, being derived from the conversion of the bicarbonate into carbonate by heat.

Precautions were taken not to overlook the excretion of unaltered bicarbonate in the urine. When the reaction of the urine is acid it is evident that there can be no bicarbonate present. In those instances where a neutral reaction was obtained, the urine was tested both before and after boiling to convert any alkali present into a form which would react more readily with litmus. Only the reaction of the first specimen voided after injection is recorded in Table II, but observations were made for at least two days after the last injection. Where "no urine" is recorded, this was determined in each instance by catheterization.

TABLE II.—*Showing tolerance for intravenous injection of alkali.*

Serial number.	Duration of illness before first injection.	Reaction of urine before injection of alkali.	Amount of alkali, first injection.	Urine.		Alkali.		Urine.		Alkali.		Urine.	
				Interval after alkali.	Reaction to litmus.	Interval between injections.	Amount, second injection.	Interval after alkali.	Reaction to litmus.	Interval between injections.	Amount, third injection.	Interval after alkali.	Reaction to litmus.
	Days, hrs.		*Grams.*	*Hours.*		*Hours.*	*Grams.*	*Hours.*		*Hours.*	*Grams.*	*Hours.*	
14	0 6	X[a]	20		No urine	4	20	15	Acid				
16	0 6	O[a]	20		do	3	40	2	Alkaline[b]	13	30	2½	Neutral.[c]
17	0 3	O	15	3	Acid	3½	20	21	Acid				
18	0 21	Acid	20		No urine	4	20		No urine	4	30	8	Acid.
19	0 8	O	40		do	6	40	7	Acid				
23	0 6	Acid	20		do	3½	20		No urine	5½	20	41	Acid.
24	0 3½	O	20		do	6	20	6	Acid				
25	0 4	Acid	40		do	7	40	17½	Alkaline				
26	0 4	O	40	2	Alkaline	6	40	8	Acid				
27	3 0	O	22		No urine	4	30	6½	do	8	30	3	Faintly acid.
28	0 9	O	30		do	4	30	22	Alkaline[d]				
39	0 7	O	30	1	Alkaline[e]	5	30	1	Acid				
47	0 13	Acid	30	9	Acid								
50	3 0	do	30	16	do								
51	1 0	O	30	40	do								
54	0 6	X	30	5	do								
55	3 0	O	30	9	do	12	30	10	Acid				
56	1 18	O	30	3	do	27	30	2½	do	7	30	2½	Acid

[a] O, signifies no urine. X, that no catheterization was made.

[b] Second specimen 2½ hours later reacted sharply acid.

[c] Remained neutral for 12½ hours after injection and then reacted acid.

[d] Second specimen 8 hours later reacted acid.

[e] Second specimen 2 hours later reacted sharply acid.

The amounts of alkali, administered as shown by the preceding table, varied from 30 to 80 grams, yet in 13 of the 18 cases the urine constantly remained acid. These data are rather definite in view of the ease with which the reaction of the urine can ordinarily be rendered alkaline, for example, by a continued vegetable diet, by the ingestion of certain organic salts, and even by a pure diuresis, such as follows the consumption of sodium chloride (7). Several of the cases also show that the intravenous injection of alkalies, at least in large amounts, will render the urine alkaline. The principal datum, then, which is required to complete this evidence of tolerance is the determination of the effect of the intravenous injection of alkalies on normal individuals, i. e., to determine whether alkalies, in relatively small quantities, when injected intravenously, affect the urine in the same manner as when they are ingested in the alimentary tract.

Absolute conditions of metabolism experiments with exact regulation of the diet were not attempted. Intravenous injections were made in normal adult Malays. The individuals selected were obtained from those classes who live largely on a vegetable diet. The same routine was followed as for the cholera cases. Sodium bicarbonate was given, in 1.5 per cent solution, using a part of the same stock which was employed for the work given in the preceding table. The bladder was emptied by catheter immediately before injection. Either a rather concentrated solution of alkali must be employed or else a relatively large amount of fluid must be introduced. In view of these disadvantages, an extensive series of normal cases was not considered advisable. Three cases only were tested.

TABLE III.—*Intravenous injections of alkali into normal individuals as a control for Table II.*

Number.	Reaction of urine before injection of alkali.	Amount of alkali.	Effect on urine.
		Grams.	
1	Acid	2.0	Reaction constantly acid.
2	do	4.5	Alkaline for 5¼ hours after injection.
3	do	5.0	Alkaline or neutral for 11½ hours after injection.

DEGREE OF ACIDITY OF THE URINE.

Titrations of the acidity of the urine could hardly give very important information concerning the acid metabolism. However, in many instances the reaction of the urine not only remained acid after the administration of the alkali, but the acidity was considerably increased, as determined by titration. Table IV shows the maximum acidity found in cases treated with chloride, bicarbonate and acetate of sodium. The estimations were made by titration with $\frac{N}{10}$ sodium hydroxide, with phenolphthaleïn as an indicator, according to the recommendations of Nægeli (8). For convenience, the data are expressed in fractions of a normal solution.

TABLE IV.—*Showing maximum acidity of urine in fractions of a normal solution after intravenous injection.*

Sodium chloride, maximum acidity of urine.	Sodium acetate.		Sodium bicarbonate.	
	Amount, in grams.	Maximum acidity of urine.	Amount, in grams	Maximum acidity of urine.
$\frac{1}{62}$	60	$\frac{1}{18}$	40	$\frac{1}{13}$
$\frac{1}{11}$	15	$\frac{1}{100}$	35	$\frac{1}{14}$
$\frac{1}{23}$	60	$\frac{1}{21}$	70	$\frac{1}{50}$
$\frac{1}{25}$	40	$\frac{1}{12}$	80	$\frac{1}{13}$
$\frac{1}{32}$	80	$\frac{1}{66}$	60	$\frac{1}{54}$
$\frac{1}{20}$	80	$\frac{1}{73}$	40	$\frac{1}{25}$
$\frac{1}{16}$	40	$\frac{1}{17}$	40	$\frac{1}{46}$
$\frac{1}{17}$	80	$\frac{1}{18}$	60	$\frac{1}{18}$
$\frac{1}{62}$	80	$\frac{1}{45}$	60	$\frac{1}{12}$
$\frac{1}{10}$	40	$\frac{1}{14}$	90	$\frac{1}{44}$
$\frac{1}{18}$	60	$\frac{1}{19}$	30	$\frac{1}{35}$
$\frac{1}{50}$	60	$\frac{1}{18}$	30	$\frac{1}{15}$
$\frac{1}{71}$	40	$\frac{1}{15}$	30	$\frac{1}{13}$

GENERAL AVERAGE.

$\frac{1}{30}$		$\frac{1}{26}$		$\frac{1}{20}$

In the preceding table, it is seen that high and low values occur in all three groups of cases. The normal range as stated by Nægeli varies from one one-hundredth to one-thirtieth; in the sodium chloride group there are several instances where the concentration is higher than one-thirtieth normal. The general averages which have been calculated have relatively little significance since many of the cases had received almost sufficient acetate or alkali to neutralize the urine. However, the behavior of the individual cases showed definitely that, *within certain limits*, the injection of bicarbonate or acetate was followed by an increase in acidity of the urine. The first or second specimen voided after injection often showed a normal degree of acidity, but the latter increased rapidly, usually reaching its maximum during the first twenty-four hours after secretion commenced. The decline to normal took place gradually, requiring two or three days. The cause of the increased acidity following the injection of alkali was not determined. One possible factor might be found in the sudden diminution of the albumen content of the urine, which sometimes occurred in cases treated with alkali. This diminution was often quite noticeable in the amount of precipitate obtained on boiling the urine. It is possible, where the albumen content is high, that considerable acid is bound as acid albumen, but with the diminution in the amount of albumen, less acid may be removed during precipitation and becomes available for the neutralization of the alkali.

The presence of alkali albumen in the urine would also be of considerable importance on account of its acid properties. Several specimens of urine were examined for alkali albumen in which the acidity, after the injection of acetate or alkali, was equivalent to one-twentieth of a normal solution or more. Eight samples were tested. In five, no precipitate was obtained on careful neutralization, and in the remaining three only a trace of turbidity appeared, which did not dissolve in excess of alkali. On adding sodium chloride to saturation, three specimens remained clear, four showed a faint trace of precipitate and in one a moderately heavy precipitate appeared. These urines of course contained more or less protein on account of the accompanying nephritis. This last specimen of urine, which precipitated with sodium chloride, gave no precipitate when the acid solution was first heated to boiling and the precipitated proteins removed by filtration.

RELATION OF THE TOLERANCE FOR ALKALIES TO THE ENTERITIS AND TO URÆMIA.

It is of considerable interest to determine whether this tolerance for alkalies depends upon the cholera infection itself or upon the accompanying nephritis. It is important to note that the tolerance apparently increases as the nephritis develops and persists after the enteritis has subsided. In Table II, the only alkaline reactions which were obtained occurred during collapse (cases numbers 16, 25, 26, 28, and 39). Of the patients receiving alkali by mouth, one case (Table I, number 1) was free from symptoms, and examination of the stool no longer showed cholera vibrios to be present; nevertheless, there were indications of a slight tolerance for alkali. A few preliminary tests were sufficient to show at least that nephritis does not always cause a tolerance.

Only four cases were tested, three of chronic and one of acute nephritis. The three chronic cases were voiding freely and showed no definite tolerance. One of these cases was given 4 grams and the other two 8 grams each of sodium bicarbonate by mouth, in one dose, and the urine promptly became alkaline in each instance. However, the case of acute nephritis had developed partial suppression of urine and was excreting about 375 cubic centimeters daily. Fairly definite signs of tolerance were obtained. The ingestion of 32 grams of sodium bicarbonate, in 8-gram doses every three hours, failed to render the urine alkaline. On the day following the administration of alkali, there was no increase in the amount of urine excreted, the twenty-four hour specimen measuring 370 cubic centimeters. On the second day after the first test, an intravenous injection of 1,000 cubic centimeters of a 1.5 per cent sodium bicarbonate solution was given according to the routine used in the cholera cases. Only one alkaline specimen of urine was obtained, and this was the first sample voided after the injection. The acid reaction of the urine returned within two hours after the injection and all succeeding specimens for the next twenty-four hours had an acid reaction. The total volume of urine for the next twenty-four hours increased to 600 cubic centimeters.

While these results are by no means conclusive, yet they might suggest that tolerance was most likely to occur in those cases of nephritis where suppression of urine has developed.

CLINICAL SIGNS OF ACID INTOXICATION.

During the stage of reaction in cholera it is not uncommon to see an almost abnormally bright color of the mucous membranes associated with a type of dyspnœa approaching air-hunger. Such patients usually have either a partial or complete suppression of urine and an increase in the blood pressure which is sometimes as high as 200 millimeters of mercury. In cholera, the symptoms of acid intoxication become so intimately related to those of uræmia that differentiation is hardly possible. Indeed it has been suggested by Senator (9) that uræmia from any cause whatsoever is only an acid intoxication.

INDICATIONS FOR TREATMENT.

The symptoms which indicate an increased production of acid in cholera are as follows:

(1) The tolerance for alkalies is well marked; (2) the ammonia coefficient is almost constantly increased; (3) clinically, there is a stage in which many cases develop an abnormally bright color associated with dyspnœa; (4) as reported in the literature, the blood shows a diminution in alkalinity.

This evidence is hardly sufficient to constitute a proof of acid intoxication. Perhaps the most important point is the increased tolerance for alkalies. Even if we assume that the alkali injected is neutralized in the body by acid, it does not necessarily follow that the acid existed preformed in the body in sufficient quantity to effect neutralization. However, in addition to this group of symptoms, the period of starvation in cholera also offers a possible etiologic factor for the production of acid intoxication. Although the period of starvation is relatively short, amounting only to a few days, yet this means not only absolute deprivation of food, but perhaps a much greater tissue waste than would occur in a normal individual when starved.

There is another explanation depending upon a somewhat different basis which should also be considered. Although alkalies are rapidly excreted by the kidneys in normal individuals, yet the anuria of cholera may permit their retention for a longer or shorter period and thereby possibly give rise to opportunities for the formation of alkali albumen. If we assume, for example, that alkali albumen is formed during a period of anuria, then even after the secretion of urine has commenced, any additional alkali which might be injected could be neutralized in the body by the acid properties of the alkali albumen already formed. The conditions bearing upon the possibility of the formation of alkali albumen were as follows: The concentrations of bicarbonate before injection varied from 1 to 2 per cent. Estimating the quantity of the blood as one-twentieth of the body weight in cholera patients, the maximum concentration of alkali after injection would be about 0.8 per cent. In certain cases where the loss of fluid had been replaced by sodium chloride solution, the quantity of blood may be estimated as normal, and the minimum concentration calculated for the 1 per cent solution of bicarbonte would be about 0.3 per cent after injection. The duration of the period of anuria was rather long in some instances, for example, fifty and forty hours in numbers 23 and 51, Table II. On the other hand, several cases which showed a definite tolerance were excreting rather freely, for example, numbers 54 and 56 in Table II. Hence, it is hardly probable that the formation of alkali albumen could be the sole factor in explaining the differences between the control individuals and the cholera cases.

Lastly, it may be noted that the condition known as acid intoxication is but imperfectly understood and that there is no standard method for its diagnosis.

The essential feature to be determined in regard to treatment, is whether this apparent tendency toward acid intoxication is a protective mechanism which should be encouraged, or whether it is a deleterious condition which should be opposed. During the intense cyanosis of collapse, the use of alkalies, theoretically, has relatively little value. On the other hand, the stage of reaction constitutes an entirely different condition. In the administration of alkalies the principal points to be determined are, namely: (1) How early does the tolerance for alkalies develop and at what stage should their administration be commenced? (2) In what form should alkali be employed and in what amount may it be used?

The symptoms of acid intoxication become more pronounced as the disease progresses. The tolerance for alkalies apparently did not reach its height before the third or fourth day. The ammonia coefficient was usually not excessively increased during the first and second day. During collapse there was intense cyanosis and the bright, flushed color did not appear until patients were well along in the stage of reaction. As to the period at which the administration of alkalies must be begun in order to prevent the appearance of acid intoxication, this must be determined by more or less empiric methods.

In the treatment of acid intoxication there are, apparently, two objects to be accomplished, namely, (1) the neutralization of acid, and, (2) the removal of carbon dioxide. Three groups of chemicals may be considered for this purpose: The normal carbonates and hydroxides, the bicarbonates, and certain neutral salts of the organic acids.

Of the first group, normal sodium carbonate is most commonly employed and, of course, without preliminary alteration combines with carbon dioxide and neutralizes acid. The bicarbonates, being saturated with carbon dioxide, can only neutralize acids, while the organic salts, such as the acetates and citrates, can do neither. However, both the organic salts and the bicarbonates are converted in the body, in part at least, to the carbonate. The ultimate decision as to the most suitable form of chemical to be employed may be found to vary according to the stage of the disease in which its administration is commenced. We have tried normal sodium carbonate, sodium bicarbonate, and sodium acetate from the three groups just mentioned. Normal sodium carbonate, in relatively small quantities, has long been recommended for use in cholera, but no reports have been found as to the effect of this treatment. Apparently the largest amount which has been used is that recommended by Manson,(10) who suggests a concentration of 60 grains to the quart, injecting about 2 to 3 quarts of fluid, making a maximum of about 12 grams of sodium carbonate. This solution is apparently intended to be used for the treatment of collapse.

EFFECTS OF ALKALIES IN ASIATIC CHOLERA.

In testing the normal carbonate, a slightly stronger solution than that used by Manson was prepared, namely, 0.5 per cent each of sodium chloride and sodium carbonate, and used in two cases *in extremis.* Following the usual routine, 2 liters of fluid were employed and in each instance death occurred five hours after the injection.

The first case was one which had been in uræmia for three days. The injection was followed, after about four hours, by slight convulsions, but it is impossible to say whether or not they were caused by the alkali. The urine did not become alkaline. The second case was in total collapse and did not respond to the injection. No urine was secreted. There was a slight muscular rigidity which lasted a few minutes. The suppression of urine could hardly have been responsible for this rigidity, since the total duration of illness was less than one day. No changes in the erythrocytes occurred in either case.

Because of the apparent tendency to cause convulsions, the normal carbonate was discarded and a small group of cases was tested, using sodium bicarbonate in varying amounts and concentrations, but always commencing the injections during the stage of collapse. In the majority of cases, two liters of fluid have been given at each injection. Three concentrations of sodium bicarbonate were employed, namely, 1, 1.5, and 2 per cent. One-half per cent of sodium chloride was added to the 1 per cent solution, but the two stronger solutions were made up without the addition of any other salts.

The preparation of sterile sodium bicarbonate in large quantities (20 to 40 liters) offers some difficulties because of the instability of this salt at the temperatures required for sterilization. The normal carbonate is rapidly formed even at 70° C. When solutions are heated in the autoclave a considerable portion of the bicarbonate is changed permanently to the normal carbonate. All of the concentrations used were sterilized by heat except some of the 2 per cent solutions, which were filtered through porcelain.

Estimation of the amount of change occurring during sterilization was made by titration against $\frac{N}{10}$ hydrochloric acid with phenolphthaleïn at a temperature of 0° C. At any temperature a solution of neutral alkali carbonate is hydrolyzed according to the equation $Na \cdot Na\,CO'_3 + H \cdot OH' \rightleftharpoons Na \cdot OH' + Na \cdot HCO'_3$ At ordinary temperatures a sharp end-point can not be obtained, but at 0° the solution may be titrated against hydrochloric acid with phenolphthaleïn to a fairly exact equilibrium. The concentration of the hydrogen ions from the dissociation of sodium bicarbonate is too low to affect the indicator and the reaction takes place just as if the latter were not present; the amount of acid required for neutralization represents, therefore, only half of the alkali present as normal carbonate. Sterilization was carried on for one hour at 7 pounds pressure in an autoclave connected with live steam. By keeping conditions constant with regard to pressure and duration of heating, successive lots of solution were obtained which were fairly constant in composition.

According to the basis of calculation just outlined the average amount of change was as follows:

	After sterilization.			
$NaHCO_3$, in grams, per 100 cubic centimeters before sterilization.	Cubic centimeters of $\frac{N}{10}$ HCl required to neutralize 10 cubic centimeters of alkali.	Per cent of $NaHCO_3$ converted to Na_2CO_3.	Grams $NaHCO_3$ per 100 cubic centimeters.	Grams Na_2CO_3 per 100 cubic centimeters.
1.0	1.8	30	0.7	0.19
1.5	2.4	27	1.1	0.26

These results do not represent a chemical equilibrium but show the progress of the reaction under certain given conditions. The fact that a relatively large amount of bicarbonate remained unchanged notwithstanding the high temperature, might be accounted for by the use of pressure during sterilization which would prevent the evolution of steam from the solution.

All the patients included in Table V, except numbers 27 and 39, were admitted in complete collapse; one case (number 27) was complicated with an acute, bloody dysentery. No detailed classification of the condition of the cases was attempted other than the separation into partial and complete collapse. Physical examination is hardly a satisfactory basis for the comparison of different cases; thus the records of the pulse, temperature, blood-pressure, respiration, and the other evidences of loss of fluid may not differ markedly in cases which evidently may have a widely different prognosis. Furthermore, cases which to all appearances are very similar may behave rather differently under the same treatment.

Seven of these 14 cases died during collapse. This is not a proportionately greater death rate from collapse than occured in the control series treated with sodium chloride; yet observation of the individual cases seemed to indicate that in some instances they responded less promptly to the alkaline solution.

The use of organic salts affords certain distinct theoretical advantages. On injection, these neutral salts possess only the properties of salt action and might be equally efficient with sodium chloride for the treatment of collapse. However, subsequently after oxidation they would afford the properties of an alkali and also of a diuretic. Furthermore, a salt can be chosen which is chemically stable during sterilization, thereby avoiding the formation of the hydroxide or normal carbonate such as occurs in the sterilization of sodium bicarbonate.

The group of cases recorded in Table VI was tested with sodium acetate. The acetate was selected in preference to the citrate because of the solubility of calcium acetate. Busquet and Pachon (11) have reported that the citrates, the normal carbonates and that group of salts which precipitates calcium are more deleterious in their action on the heart than those acids, the calcium derivatives of which are soluble. The concentrations given in Table VI are calculated for the crystallized salt, the 1.5 per cent solution representing approximately 0.9 per cent and the 2 per cent solution 1.2 per cent of the anhydrous salt.

The patient (number 32) who died in uræmia represented a type of those cases which develop only a mild enteritis, but terminate in a fatal uræmia.

This patient, on admission, received 30 grams of sodium acetate intravenously. The first laboratory examination did not show cholera vibrios in the stool. The condition of the patient appeared altogether favorable, but on the fifth day, slight symptoms of uræmia developed in addition to the partial suppression of urine which had been present, and a second examination of the stools showed the presence of cholera vibrios. On the sixth day, 30 grams of sodium bicarbonate were injected intravenously. The secretion of the urine started promptly and in the following fifteen hours 600 cubic centimeters were excreted, an amount equal to the total volume for the five days previously. The urea content was 7.8 grams as compared with 1.9 grams for the same volume of urine excreted before the injection of alkali.

In another instance the acetate was discontinued after the first injection. Symptoms of uræmia developed rather suddenly, and injection of sodium bicarbonate was commenced on the third day. In the remaining twelve days of life, a total of 180 grams of sodium bicarbonate was

TABLE V.—*Cases treated during collapse with sodium bicarbonate.*

Serial number.	Age.	Duration of illness.		Cubic centimeters of urine on admission.	First day.			Second day.			Amount of urine, in cubic centimeters.			Reaction of urine to litmus.	Total amount of alkali, in grams.	Length of course of the disease.		Termination.
					Sodium bicarbonate.		Cubic centimeters of urine.	Sodium bicarbonate.		Cubic centimeters of urine.	Third day.	Fourth day.	Fifth day.					
					Percent.	Amount, in liters.		Percent.	Amount, in liters.									
		Days.	*hrs.*													*Days.*	*hrs.*	
11	25	0	8	7	1	4	0								40	0	16	Death; collapse.
14	25	0	6	X[a]	1	4	0			380	1,580	1,600	1,690	Acid	40	15	0	Recovery.
16	30	0	6	0	1 2	2 3½	40			1,020	575	650	1,065	Alkaline	90	19	0	Do.
17	35	0	2½	0	1	3½	2			540	505	680	1,270	Acid	35	18	0	Do.
18	19	0	21	1	1 2	4 1½	100			0				do	70	1	16	Death; collapse.
19	38	0	8	0	2	4	40			700	1,740	1,050	330+	do	80	6	0	Death; pneumonia.
21	40	0	6½	0	2	3½	0	2	1½	0					100	2	0	Death; collapse.
23	56	0	6	10	1	6	0	(b)	(b)	0	85	815	2,170	Acid	60	17	0	Recovery.
24	28	0	3½	0	1	4	10			135	20	655	710	do	40	17	0	Do.
25	50	0	4	5	2	4	0			5				Alkaline	80	0	24	Death; collapse.
26	18	0	4	0	2	2	10			30				do	40	0	21	Do.
27	30	3	0	0	1½	5½	140							Acid	82	4	0	Death; enteritis.
28	19	0	6	0	1½	4	0			60	255	1,030	2,650	Alkaline	60	16	0	Recovery.
39	21	0	7	0	1½	4	135			40	845	1,530	2,180	do	60	17	0	Do.

[a] No catherterization.

[b] Normal salt solution, 2 liters.

TABLE VI.—*Cases treated with sodium acetate.*

IN PARTIAL COLLAPSE.

Serial number.	Age.	Duration of illness.		Cubic centimeters of urine on admission.	First day.			Second day.			Amount of urine, in cubic centimeters.			Reaction of urine to litmus.	Total amount of acetate, in grams.	Length of course of the disease.	Termination.
					Acetate.		Cubic centimeters of urine.	Acetate.		Cubic centimeters of urine.	Third day.	Fourth day.	Fifth day.				
					Percent	Amount, in liters.		Percent.	Amount, in liters.								
		Days.	*hrs.*													*Days.*	
32	29	0	7	0	1.5	2	2			50	270	200	[a] 80	Acid	30	7	Death; uraemia.
33	25	0	14	0	2	2	0			830	1,050	1,300	1,300	do	40	10	Recovery.
37	40	0	10	0	2	[b] 4	0			0	70	140	1,800	do	80	12	Do.
43	27	0	10	0	2	2	0			110	465	1,580	1,700	do	40	13	Do.
44	30	0	7	0	2	[b] 4	0			330	140			do	80	4	Death; enteritis.
38	18	3	0	0	(b)		0	2	4	0	280	830	720	do	80	9	Do.
IN COMPLETE COLLAPSE.																	
30	28	0	16	0	1.5	2	0	1.5	2	0	390	950	1,450	Acid	60	13	Recovery.
31	57	0	6	0	1.5	4	0			20	(c)	(c)		do	60	9	Do.
34	60	0	7	0	2	4	0			100	950	1,400	1,000	do	80	9	Do.
35	55	0	24	0	2	[b] 4	0			10	1,300	560	650	do	80	9	Death; pneumonia.
36	40	0	10	x	2	[b] 4	0			0	15			Alkaline	80	3	Death; enteritis.
42	28	0	4	0	2	[b] 4	0			0	740	2,250	1,280	Acid	80	10	Recovery.
46	22	0	3	0	2	4	5	(b)		0	260	(c)	2,100	do	80	14	Do.
49	50	0	17	10	2	2	100	2	2	30	440	1,250	470	do	60	8	Death; enteritis.

[a] On the sixth day this patient received 30 grams of sodium bicarbonate intravenously.

[b] Two liters of isotonic salt solutions were also given following the injections of sodium acetate.

[c] Involuntary.

There were also 4 cases dying in collapse after an illness of less than two days and a fifth case which is described in the text.

injected. The secretion of urine commenced promptly after the first injection and continued until death. The blood-pressure, after the first injection, fell from 140 to 115 millimeters of mercury. The color continued very bright and the respirations were deep, but the rate was normal. On the eleventh day of the disease, examinations of the stools showed them to be free from vibrios. Death occurred four days later. At autopsy the small intestine was found to be almost gangrenous. There was no attempt at reparation of the intestine, the lower half of the ileum being almost entirely denuded of the mucosa. In some areas, only the muscularis and serous coats remained.

The total duration of the disease in this patient is in marked contrast with the series of cases treated with sodium chloride in which seven days was the maximum course for the fatal cases.

If the percentage of mortality is considered in this group, it must be noted that four of the cases were almost moribund on admission. Occasionally, the secretion of urine did not commence very promptly after the injection of acetate. After secretion was established, the amount voided was usually about normal, although the nitrogen output as judged by the urea content was frequently rather low. However, some of the cases with low urea output made very satisfactory recoveries. Although in normal individuals acetates are oxidized almost completely to alkali, yet it does not follow that cholera patients would effect the complete oxidation of excessively large quantities of acetate. However, no acetate could be detected in the urine by the ferric chloride or ethyl acetate tests, although these reactions were rather unsatisfactory when applied to urine. In three cases where the acetate was given in 80-gram quantities, the distillation of the strongly acidified urine showed only a trace of volatile acid.

A third group of cases was studied, abandoning any attempt at a routine for all patients. The general plan of treatment was to use sodium chloride solution for the stage of collapse, starting the sodium bicarbonate as soon as the stage of reaction commenced. The bicarbonate was used when indicated for patients who did not go into collapse, its indication being judged by the dyspnœa, the flushing of the cheeks and mucous membranes, the suppression of urine and the rise of blood pressure. For those cases where the loss of fluid was moderate and it seemed probable that only one or two injections would be required, the bicarbonate was used for the first injection.

In addition to the cases recorded in Table VII, there were also five other cases which were placed in the bicarbonate series on admission. These died under sodium chloride treatment before the stage of reaction was reached; consequently they received no alkali, as there was no definite indication for its use. In the outline which follows later, these five cases are included with the number of deaths from collapse occurring under alkaline treatment, for otherwise the alkali series would represent selected cases.

TABLE VII.—*Cases treated both with sodium chloride and sodium bicarbonate.*

IN PARTIAL COLLAPSE.

Serial number.	Age.	Duration of illness.		Cubic centimeters of urine on admission.	First day.			Second day.			Third day.			Amount of urine, in cubic centimeters.		Reaction of urine to litmus.	Total amount of alkali, in grams.	Course of the disease.	Termination.
					Sodium bicarbonate.		Cubic centimeters of urine.	Sodium bicarbonate.		Cubic centimeters of urine.	Sodium bicarbonate.		Cubic centimeters of urine.	Fourth day.	Fifth day.				
		Days.	*hrs.*		Percent.	Amount, in liters.		Percent.	Amount, in liters.		Percent.	Amount, in liters.						*Days.*	
47	30	0	8	40	1.5	2	100			1,240			4,660	3,880	1,240	Acid	30	8	Recovery.
48	60	0	9	0	1.5	[a]2	0	1.5	2	0			[b]215	600	760+	Alkaline	90	15	Do.
50	25	2½	0	30	1.5	2	0			320			2,750	2,800	960+	Acid	30	15	Do.
52	45	0	2	0	(c)		20			100	1.5	2	230	1,290	3,090	do	30	21	Do.
55	68	0	11	0	(c)		0			10			13	[d]135	1,580	do	60	17	Do.
56	32	0	9	0	(c)		20	1.5	2	15	1.5	4	1,820	6,630	2,840+	do	90	6	Death; pneumonia.
59	23	2	0	×	1.5	2	0			1,050			1,240	Convalescent.		do	30	10	Recovery.
63	30	0	16	×			0	1.5	2	105	1.5	2	370			do	60	3½	Death; enteritis.
IN COMPLETE COLLAPSE.																			
51	49	1	0	0	1.5	2	0			68			1,460	1720	1,440	Acid	30	20	Recovery.
54	19	0	6	×	1.5	2	20			1,920			1,460	1230	1,055	do	30	20	Do.
57	48	0	9	0	(c)		0	(e)		0	1.5	2	140	[d]865	(f)	do	90	9	Death; enteritis.
58	65	1	0	×	(c)		0	1.5	2	0	1.5	2	175	240	1,000	do	60	9	Recovery.
60	30	0	9	0	(c)		0	[e]1.5	2	0	(e)		30	0	10	do	30	4	Death; enteritis.

61	30	0	14	×	1.5	2	0	1.5	2	0	----	----	0	[b]0	2,730	do	90	17	Recovery.
62	30	0	7	0	([c])	----	0	1.5	2	1,320	----	----	2,350	1990	1,220	do	30	11	Do.
64	40	1	0	0	([a])	----	0	([d])	----	0	1.5	2	140	[b]780	1,510	do	60	15	Do.
65	37	3	0	×	1.5 ([e])	2	50	----	----	----	----	----	----	----	----	do	30	4	Death; collapse.

[a] Two injections isotonic salt solutions 2 liters each.

[b] Two liters of 1.5 per cent of sodium bicarbonate.

[c] Hypertonic salt solution 2 liters.

[d] Four liters of 1.5 per cent of sodium bicarbonate.

[e] Three injections isotonic salt solutions 2 liters each.

[f] Involuntary.

This group of cases showed that the injection of bicarbonate, in sufficient quantity, was followed by an active secretion of urine. In one case (number 56) excessive amounts were voided, more than 6 liters being excreted in twenty-four hours. The urine secreted after the injection of alkali differed in several respects from that obtained after the injection of the chloride; after the use of the bicarbonate, both the quantity of urine and also the urea content (see Table IX) rose rapidly to normal. The acidity as determined by titration was sometimes increased. Not infrequently the amount of albumen diminished rapidly and the urines became albumen free at an early period.

One patient (number 50) in this series was of special interest.

He was admitted after an illness of two and one-half days. The symptoms of uræmia were fairly definite. The suppression of urine was absolute. Respiration was natural. The cheeks were flushed and the mucous membranes were bright pink in color. The pulse was full and bounding and the blood pressure measured 175 millimeters of mercury. Thirty grams of sodium bicarbonate were injected intravenously. The bright color of the mucous membranes persisted, but the tension of the pulse diminished rapidly and, after an interval of ten hours, the blood pressure had fallen to 135 millimeters. Sixteen hours after the injection, the secretion of urine commenced. The amount was small at first but steadily increased, and a prompt recovery followed.

In another patient (number 48) the anuria persisted until the third day. The secretion of urine finally commenced eighteen hours after a subcutaneous injection[4] of 30 grams of sodium bicarbonate. The first specimen of urine was entirely colorless and contained no trace of urea or ammonium salts. However, there was considerable alkali present, amounting to about one-fifth of a normal solution, which was present both in the form of carbonate and bicarbonate. The second specimen, a few hours later, contained a little pigment and a trace of urea, while the third specimen was almost normal.

In addition to following the reaction of the urine, estimations of the ammonia coefficient[5] were made as a further aid in determining the most desirable dosage of alkali. High coefficients were found, not only in the cases treated with chloride solutions, but these high values sometimes persisted even after the administration of relatively large amounts of alkali. The quantities of alkali stated in Table VIII were injected before the urine was voided.

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[4] The danger of abscess formation and necrosis after the subcutaneous injection of alkalies has been emphasized repeatedly, but this method may occasionally be justifiable in the extreme conditions of cholera. In five instances where the available number of superficial veins was limited, sodium bicarbonate was injected subcutaneously in 1.5 per cent concentration. In no instance was there any evidence of necrosis or abscess formation. Two of these cases were in an extreme condition and it was expected that a second injection of alkali would be required. A few hours after the injection, both patients complained of muscular cramps throughout the body. The secretion of urine commenced without further use of alkali.

[5] It gives me much pleasure to acknowledge the very helpful coöperation of Mr. George F. Richmond, chief of the chemical laboratory, Bureau of Science. These determinations were made under his direction, by Folin's (12) method.

TABLE VIII.—*Showing ammonia coefficient under different conditions.*

CASES TREATED WITH SODIUM CHLORIDE.

Serial number.	Amount, in grams.	Day of disease.	Nitrogen as ammonia in per cent of total nitrogen.	Day of disease.	Nitrogen as ammonia in per cent of total nitrogen.	Termination.
8		Third	15.8	Seventh	27.8	Recovery.
13		Second	12.5	Third	9.2	Do.
15		First	9.4	Second	9.9	Do.
20		do	2.5	do	20	Death; collapse.
22		Fourth	37.9	Sixth	12.5	Death; uræmia.
29		Third	0.8			Recovery.
40		Third[a] Fourth Fifth			12.2	Death; uræmia.
41		Fourth	8.5			Recovery.
53		Third	25.7	Fourth	21.7	Do.
66		Second	4.2			Death; collapse.

CASES TREATED WITH SODIUM ACETATE.

30	60	Fourth	8.3			Recovery.
31	60	do	7.4			Do.
33	40	First	12.5	Second	16.3	Do.
34	80	Fifth	6.3			Do.
38	40	do	15.6			Death; enteritis.
42	80	Fourth	1.4			Recovery.
43	80	do	10.0			Do.

CASES TREATED WITH SODIUM BICARBONATE.

14	40	Second	10.8	Third	5.4	Recovery.
16	70	do	1.2	Fourth	7.3	Do.
17	35	do	16.1	Third	12.8	Do.
18	70	do	5.3			Death; collapse.
19	80	First	3.6	Second	5.5	Death; pneumonia.
23	60	do	40.7	Fourth	42.0	Recovery.
24	40	do	5.7	do	7.5	Do.
27	52	Second	5.9			Death; enteritis.
28	60	do	8.2	Fourth	8.5	Recovery.
48	30	Fourth	18.0			Do.
50	30	do	16			Do.

[a] Composite sample.

In the bicarbonate series, with one exception, only those solutions were free from normal carbonate which were sterilized by filtration without heat. There is the possibility, of course, that in the heated solutions the active agent was not the bicarbonate, but the normal carbonate which was also present. However, this explanation is excluded, first by the similarity in the action of the neutral sodium acetate as compared with the bicarbonate, and, secondly, by the fact that in two cases (numbers 27 and 28) the normal carbonate was removed from the bicarbonate solution by the following procedure:

A 2 per cent solution of sodium bicarbonate was sterilized by heat and the normal carbonate removed by adding an excess of normal hydrochloric acid. Sufficient acid was added to reduce the content of sodium bicarbonate to 1.5 per cent and the solution was then allowed to stand several hours to permit the escape of the undissolved carbon-dioxide, but no special precautions were taken to remove the carbon dioxide in solution.

CASES TREATED WITH SODIUM CHLORIDE.

For comparison with the cases treated with alkali and acetate, a duplicate series was carried out, using the ordinary treatment with sodium chloride. The general nature and severity of the cases varied comparatively little throughout the period of investigation, thereby facilitating the division of the patients into fairly comparable groups. Of the total of 111 cases investigated, the first 20 were treated with Ringer's solution. In the remaining 36 cases of the control series, sodium chloride alone was used, and these cases were distributed uniformly throughout the series. Both hypertonic (1.3 per cent) and isotonic (0.85 per cent) salt solutions were employed.[6] The following outline gives a summary of the causes of death in the group of cases treated with sodium chloride in comparison with those receiving alkali.

Treatment.	Number of cases dying in—		
	Collapse or enteritis.[a]	Pneumonia.	Uræmia.
Chloride	30	2	8
Acetate or bicarbonate	24	3	1

[a] The term "enteritis" is used to designate those cases which died from the toxæmia of the cholera infection in distinction from the toxæmia of uræmic origin.

The causes of death, usually, were rather sharply differentiated. The uræmic cases, in addition to dyspnœa and suppression of urine, showed at least a normally bright color and usually considerable elevation of the blood pressure, which sometimes registered as much as 140 to 180 and even 200 millimeters of mercury. Constipation in these cases was not uncommon in the later stages of the disease. Those patients who lived three or four days, but with cyanosis, soft pulse, and normal or subnormal blood-pressure, are considered as having died of enteritis rather than uræmia, even though respiration was labored and the suppression of urine was absolute. The initial loss of fluid by rectum in these cases might account for the absence of urine; also in these cases the diarrhœa frequently continued until death.

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[6] The conclusions in regard to the relative value of isotonic and hypertonic salt solutions for the treatment of collapse will appear in this number of the *Journal*.

In reviewing the two groups it is noteworthy that the majority of patients who recovered under the sodium chloride treatment did not develop absolute suppression of urine for as long a period as twenty-four hours. However, in the alkali series, in addition to those which did not develop complete suppression, a considerable number recovered even after two days of absolute suppression.

The following outline gives a summary of the cases which recovered.

Treatment.	Number of days of absolute suppression.			
	None.	One.	Two.	Three.
Chloride	9	6	1	0
Bicarbonate and acetate	7	12	7	1

The final results were as follows:

Treatment.	Number of cases.	Deaths in uræmia.	Total number of deaths.	Number of recoveries.
Chloride	56	8	40	16
Bicarbonate and acetate	55	1	28	27

Calculation of percentages upon such a small group of patients could have little significance. However, it is noteworthy that the increased number of cases recovering under the alkaline treatment corresponds roughly to the number of deaths from uræmia under the sodium chloride treatment. Assuming that alkalies affect uræmia only and have no influence upon collapse, then the theoretical limits of this increase would be the percentage of cases which ordinarily die in uræmia. Of the 56 cases treated with sodium chloride, 8, or 14 per cent, died in uræmia. This corresponds closely to the conditions in India where Rogers (1) reports an incidence of 13.2 per cent of uræmia in cholera.

SUPPRESSION OF UREA.

After secretion of the urine was established, determinations of the urea content showed a suppression of urea somewhat comparable to the suppression of urine. In the following table, the determinations were made by the sodium hypobromite method and therefore include the ammonium salts as well as the urea. The analyses were at first made on twenty-four hour specimens of urine, but, after a marked suppression of urine, a sudden increase in the urea content frequently occurred such as would be overlooked in estimations of the total urea excreted per day. For example, the first 100 cubic centimeters of urine voided after a period of suppression might contain only 0.3 per cent of urea. For the remaining twenty-four hours, perhaps a liter of normal urine might be voided. It was necessary to examine each successive specimen separately in order to detect these critical changes.[7]

The percentage of urea, apparently, was almost independent of the quantity of urine which was voided, and, at least as shown by Table IX, the sudden increase in percentage was not due to a diminution in the volume excreted, but was rather accompanied by an increase in volume. The changes in the urea content did not consist in fluctuations from small to large amounts, but the increased outputs were maintained.

TABLE IX.—*Showing changes in excretion of urea.*

Serial number.	Treatment.	First day.	Second day.	Third day.	Fourth day.	Fifth day.	Sixth day.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
9	Sodium chloride	0.4	0.5	0.4		1.2	
12	do	2.8		3.5			
13	do	0.4	0.35	1.1	1.5	2.4	
15	do	1.0	1.8				
22	do		0.2	0.8	0.25	0.5	0.3
23	do	0.1	0.25	0.6	0.5		
52	Sodium bicarbonate, 30 grams.	0.05	0.75	1.5			

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[7] The accurate collection of an extensive series of specimens in the midst of an epidemic was made feasible by the very excellent supervision of Miss A. E. McEvoy, Mrs. K. E. Taulbee, and Miss B. M. Gertsch, the nursing staff of San Lazaro Hospital.

TABLE IX.—*Showing changes in excretion of urea*—Continued.

Serial number.	Treatment.	Urea.	Interval.	Urea.	Interval.	Urea.	Interval.	Urea.	Interval.	Urea.
		Per ct.	*Hours.*	*Per ct.*	*Hours.*	*Per ct.*	*Hours.*	*Per ct.*		*Per ct.*
20	Sodium chloride	0.1	5	0.1	5	0.1	6	0.1		
29	do	0.4	19	0.4	Quantity about 2.5 liters daily.				Recovery	
40	do	0.2	13	0.1	12	0.15	24	0.4		
45	do	0.15	5	0.15	4	0.2	3	0.5	24 hours	0.4
19	Sodium bicarbonate, 80 grams.	0.05	16	1.2	24	1.8			After 3 days.	1.4
47	Sodium bicarbonate, 30 grams.	[a]0.3	19	1.3						
46	Sodium bicarbonate, 90 grams.	None	17	0.25	8	0.4				
50	Sodium bicarbonate, 30 grams.	[a]0.05	9	0.4	9	1.3				
54	do	0.05	2½	0.5	1	0.8	1½	1.3		
56	Sodium bicarbonate, 60 grams.	0.6	6	1.3						
33	Sodium acetate, 40 grams.	0.05	6	0.35	8	2.2				
35	Sodium acetate, 80 grams.	0.05	24	1.4						
37	do	0.4	17	1.4						

[a] Before injection of alkali.

In those cases in the sodium chloride group in which the percentage of urea was definitely lowered, the return to normal took place gradually (cf. especially numbers 53, 20, 40, and 45), while in the alkali group, a sudden increase in the output of urea was frequently noted (e. g., numbers 19, 47, 50, and 54). It would be of interest to determine whether the suppression of urea was due to the inability of the kidney to excrete urea or whether the metabolism was at fault and the organism unable to produce urea. It would seem a little unusual for the kidney to excrete fluid and salts freely, and yet be limited to subnormal amounts of urea. *A priori*, one would expect that the tolerance for alkalies is produced by a disturbance in metabolism rather than by a local lesion of the kidney. Assuming the presence of an acid intoxication, it might be expected that in the cases treated without alkali the nitrogen, which would ordinarily go to form urea, is utilized for the neutralization of acids, but in the presence of alkali it is no longer needed for this purpose and is excreted as urea.

CONCLUSIONS CONCERNING TREATMENT.

The behavior of these various groups of cases has led to the following conclusions:

1. The choice of alkali for treatment may vary somewhat in the different stages of cholera. The chief advantage of the normal carbonate would depend upon its ability to absorb carbon dioxide, but apparently few deaths occur primarily from failure of the internal respiration.

2. Sodium acetate may have some advantages, especially for the stage of collapse. As much as 80 grams within twenty-four hours have been injected, but perhaps this amount is slightly excessive in certain cases.

3. As a general routine, sodium bicarbonate has been the most effective of the three salts. Its administration in collapse has several advantages and, in selected cases, concentrations of 1 or 1.5 per cent may be used. A weaker solution could probably be chosen which would be suitable for routine use in all cases of collapse. Early in the stage of reaction, at least as much as 60 grams may readily be given within twenty-four hours. The most important indication for discontinuing its administration is the development of muscular cramps or twitchings. These symptoms may appear, although the urine remains acid, and may be considered as a reaction to the alkali. Several cases in which excessive amounts of alkali were tolerated without the appearance of this symptom, terminated unfavorably. There was a wide variation in the amount of alkali which resulted in the production of muscular contractions. The quantity apparently varied in direct proportion to the severity of the disease although Loeb (16) has shown that the precipitation of the calcium salts by carbonates gives rise to muscular contractions. If administration of alkalies is delayed until uræmic symptoms develop, secretion of urine follows promptly after injection of sodium bicarbonate, but the final termination is usually unaltered.

4. In no case was the urine kept constantly alkaline. Indeed, in the majority of cases an alkaline reaction was never obtained, and it is difficult to conjecture what the effect might be of maintaining a constantly alkaline urine throughout the course of the disease.

5. The most important single factor in determining the amount and frequency with which alkali should be injected is the quantity of urine excreted. Sodium bicarbonate in 1.5 per cent solution in 2-liter quantities has been injected as soon as patients come out of collapse and repeated at twelve to twenty-four hour intervals until a free secretion of urine follows. Aside from mild convulsions, the only other untoward symptoms following the injection of alkali was a temporary hæmaturia. This occurred in 3 of the 55 cases. It was slight in amount and persisted for from two to four days.

6. In comparing the two groups of cases, namely, those treated

with chloride and those with alkali, the most important difference is the absence of uræmia in the bicarbonate series. As a rule, morphia failed to quiet patients when the stage of uræmia had developed. However, after the injection of alkali, the respiration improved and the restlessness of the patients disappeared. Observation of the individual cases sometimes showed a sudden improvement following the injection of alkali. The value of the injection of alkalies is seen most clearly in that class of cases which have only mild symptoms of cholera, but which nevertheless develop a fatal uræmia.

7. In the specific treatment of cholera, there are several distinct pathologic conditions which are commonly present and, consequently, no one treatment can meet them all. The treatment of the toxæmia, which is the most important factor, is not under satisfactory control. The effect of fluid on the relief of mechanical conditions in the stage of collapse is well established. Symptoms of acid intoxication and the development of uræmia during the stage of reaction did not occur (except in one instance) in a group of fifty-five cases treated with acetate or bicarbonate of sodium.

GENERAL SUMMARY.

1. Examination of the urine in cholera showed an almost constant increase in the excretion of ammonia.

2. Cholera patients showed a definite tolerance to alkalies, a considerable excess of sodium bicarbonate being required to render the urine alkaline as compared with normal individuals. Within certain limits, the administration of alkalies not only failed to render the urine alkaline, but its acidity was even increased, as measured by titration. Following the injection of alkalies, there was sometimes a sudden and marked increase in the excretion of urea.

3. The early administration of alkalies practically eliminated death from uræmia.

The most important findings are the tolerance for alkalies and the change in the course of the disease. This tolerance may be dependent either upon the cholera infection, or upon the accompanying nephritis; apparently it is related more closely to the uræmia than to the enteritis. The condition will be of more general interest if it proves to be present in uræmia from other causes than if it is specific for cholera.

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EFFECT OF THE CONCENTRATION OF SOLUTION IN THE TREATMENT OF COLLAPSE IN ASIATIC CHOLERA.

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One of the most prominent features of Asiatic cholera is the extremely rapid secretion of large amounts of fluid by the intestine during the early stages of the disease. The excretion of water by the ordinary channels practically ceases. The skin usually becomes dry and the urine is partially or completely suppressed. In cases of average severity this constant flow of fluid from the blood and other tissues into the intestines ceases only when there is no more available fluid in the body, and it usually recommences when liquid is supplied. This process might be regarded as a compensatory reaction for removing bacteria and their products and at the same time rendering conditions unfavorable for absorption from the intestine. Dangerous symptoms appear only when the supply of fluid fails, and these symptoms are relieved, at least temporarily, by the restoration of fluid to the body. Naturally, the restoration of fluid becomes an important matter and the various methods employed to accomplish this have attracted considerable attention. Sodium chloride is the solution which is in common use, and the concentrations employed usually have been divided under a physiologic classification into three groups, namely, (1) hypotonic, (2) isotonic, and (3) hypertonic solutions. The theoretical value of these three concentrations may briefly be outlined as follows: The hypotonic solution, by reason of its subnormal osmotic pressure, would tend to continue the excretion of water from the vascular system into the intestine, necessitating, perhaps, the frequent injection of relatively large amounts of salt solution. The hypertonic solution would tend to restore water to the body with a minimum loss of injected fluid. By reason of the higher osmotic pressure of the hypertonic solutions the loss of fluid from the blood vessels into the surrounding tissues might be delayed, thereby checking the diarrhœa. This increase of tonicity, if it were sufficiently pronounced, might even result in absorption of fluid from the intestine. This, of course, would be accompanied by the possibility of the absorption of bacterial products. However, in practice, it is not supposed that the solutions employed are sufficiently strong to cause any such harmful action. The effect of the isotonic solution would fall between these two extremes, and presumably it would not interfere with the natural processes of the body. The essential question is whether one should attempt to maintain the circulation with the use of as little fluid as practicable, checking the diarrhœa if possible, or whether liberal amounts of fluid should be used, allowing the diarrhœa to continue. In some respects the absolute cessation of excretion by all channels is less desirable than a limited amount of diarrhœa.

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Hypertonic salt solution has been in use for several years in the treatment of the stage of collapse in cholera, but Rogers recently has used it extensively and has reported very favorable results. He compared the results obtained with hypertonic solution of 1.3 per cent with those obtained with a hypotonic solution of 0.6 per cent.

In the autumn of 1909 a small epidemic of cholera occurred in Manila and at the request of Doctor Strong the effect of different concentrations of salt solution for treatment were again studied. Two solutions were tested, an isotonic one of 0.85 per cent and a hypertonic of 1.3 per cent. The first 10 cases in each series were treated with a Ringer's solution which was prepared according to the following formula: Sodium chloride, 0.8 per cent; potassium chloride, 0.04 per cent; and calcium chloride, 0.025 per cent for the isotonic and for the hypertonic solution, the sodium chloride was increased to 1.3 per cent, but the calcium and potassium salts were not changed. For the remaining cases sodium chloride only was used, in 0.85 per cent and 1.3 per cent solution.

In comparing different concentrations it is important, of course, to know the changes in the salt content of the blood. In the following work it has been assumed that the loss of salts occurred somewhat proportional to the loss of fluid and that the relative differences existing in the two salt solutions were not fundamentally altered after injection.

The cases were divided into two series as nearly comparable as possible. One of these series received isotonic and the other hypertonic salt solution. As a routine, 2 liters of fluid were injected intravenously in the course of one-half hour. The number and frequency of the injections was varied according to the individual case. In general, they were repeated when the pulse became weak and rapid and when the blood pressure registered between 50 and 70 millimeters of mercury.

Measurements of the blood pressure were made on all cases. Rising frequently from zero, the maximum was reached about one to three hours after the injection. This maximum varied somewhat in individual cases, but usually amounted to a slight rise above normal.

There was no apparent difference in the frequency with which the injections were required to be repeated in the two series of cases. The physical signs indicating the absorption of fluid by the tissues were quite similar after the use of either solution. Usually, after the second or third injection, the shrinking of the fingers disappeared, the skin over the extremities resumed its elasticity and the power of speech returned.

Neither the course of the diarrhœa nor the height of blood pressure which was obtained, bore any apparent relation to the solution which was employed. The following outline gives a general summary of the two groups.

Treatment.	Average number of injections.	Average maximum blood pressure after injections.	Deaths.						Total deaths.	Total recoveries.
			Collapse.				Uræmia.	Pneumonia.		
			First day.	Second day.	Third day.	Total.				
Isotonic	2	128	7	7	4	18	3	1	22	9
Hypertonic	1½	121	4	8	5	17	5	1	23	7

As regards the secretion of urine, no differences were observed. The proportion of cases dying in uræmia was practically unaffected by the use of the hypertonic salt solution. This agrees with Rogers' results.

In addition to the cases treated with hypertonic solutions of sodium chloride, a series of patients was treated in this hospital with hypertonic solutions containing relatively large amounts of sodium bicarbonate. This salt is of especial interest in the consideration of hypertonic solutions, since, in concentrated form, the bicarbonates have the property of withdrawing fluid from the tissues. Twelve cases admitted at the hospital received the alkaline hypertonic solution during the stage of collapse. The comparison of the three solutions in the treatment of collapse is as follows:

Solution employed.	Number of cases.	Death in collapse.	
		Number.	Per cent.
Neutral isotonic	31	18	58
Neutral hypertonic	30	17	57
Alkaline hypertonic	14	7	50

However, the effect of the alkaline solution is not merely a question of hypertonicity. In addition to its property of attracting fluid from the tissues, it also has diuretic properties. These characteristics may be of equal importance with the ionic concentration of the solution in the interpretation of the treatment of collapse. A detailed report of the series treated with alkali appears in the present number of the JOURNAL.

Records of the blood pressure were of interest in connection with the development of uræmia. In some cases, the blood pressure remained about normal during the stage of reaction, urine was excreted freely and recovery usually followed. However, in another class it rose rapidly during the stage of reaction, the excretion of urine was limited in amount or lacking altogether, and a fatal uræmia usually followed.

In severe cases which recovered, the urine frequently diminished in volume about one-half toward the end of the first week, or early in the second week, of a normal convalescence. This fact is of considerable importance because of the prognostic value of the output of urine.

SUMMARY.

1. No definite differences for the two concentrations of salt solution which were tested for treatment, were observed, either upon the course of the disease or in its final termination.

2. Measurements of the blood pressure were of some service in determining the frequency with which repetitions of the injections of salt solution should be given.

In considering these results and conclusions, it is important to emphasize the severity of the cases under observation. The patients were often admitted to the hospital in complete collapse and, perhaps, were not particularly suited for such an investigation, since in many instances the beneficial effects following the usual treatment with ordinary salt solution were not obtained. Also, the total number of cases (61) was small and observations upon a larger series of patients under more favorable conditions might possibly give greater differences in the effect of the various concentrations of salt solution.

THE CHEMICAL COMPOSITION OF THE BLOOD IN ASIATIC CHOLERA.

By HANS ARON.

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All attempts to counteract the action of the toxins of the cholera vibrio, directly or indirectly, have thus far failed to give satisfactory results in the treatment of Asiatic cholera. There is at present only one method which is of distinct value in this disease, namely, the injection of large quantities of saline solution. Of course, this treatment is only symptomatic, tending to replace the great loss of water, but the curative action of its replacement is so great that without doubt we must regard loss of water as one of the main factors producing the grave results of the disease. The idea is a very old one that loss of fluid from the body is one of the most important factors in Asiatic cholera. The autopsy findings, such as a dry skin, peritoneal cavity and pericardial sac, as well as the increased viscosity of the blood, all confirm this belief. Investigators have repeatedly considered the question as to the effect of a great loss of fluid on the composition of the tissues of the body and of the extent to which changes in the composition of the tissues and fluids of the body are concerned in producing the stage of collapse and early death in Asiatic cholera. The earliest and most authoritative investigations of the chemical composition of the blood were made in 1850 by C. Schmidt[1] in Dorpat. However, Schmidt's conclusion that there is a constant withdrawal of water from the blood in stages of collapse has been doubted by several authors because he used too few control subjects. The following question also frequently has been discussed during recent years: Does the blood, in addition to the loss of water, also suffer a loss of salts, and, if so, to what degree? The answer is without doubt of great importance in the treatment of Asiatic cholera, since, if water alone is lost from the blood, then we need only to replace it; but if this is true of salts as well, then the latter must also be restored by the injection of an equivalent amount.

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[1] Zur Characteristik der epidemischen Cholera gegenüber verwandten Transsudationsanomalien. Leipzig, 1850.

Two years ago, Rogers[2] stated that there is not only a great loss of water in cholera, but, especially in severe cases, of the salts (chlorides) in proportionally greater amounts than water, the blood becoming hypotonic. Therefore, he suggested the injection of hypertonic saline solution and not of a normal solution such as is usually employed. In recent papers[3] he describes very favorable results by this method of treatment. As already mentioned, Rogers bases his treatment on the fact that he found a diminution of the chlorides in the blood. The accuracy of his chemical method of estimating the content of chlorides in the blood or blood serum is open to criticism; furthermore, his differences depend upon results showing that the blood of the average healthy Bengalese contains relatively more chlorides than that of Europeans.

I have undertaken a number of further analyses because of the important bearing which the composition of the blood in cholera may have upon the pathogenesis of the disease and its successful treatment, and I began with a number of cholera patients who were brought to the San Lazaro Hospital for Infectious Diseases.

Blood samples were taken from a vein in the arm of the patient before treatment had been instituted. It is often quite difficult and sometimes impossible, especially in the stage of severe collapse, to secure even a few drops of blood in this way. In addition to the above, material was not abundant this year because the incidence of the disease in the city of Manila was very small, only two or three cases occurring per day, and a number of these were children. All the patients from whom blood was taken showed the typical picture of cholera, and the clinical diagnosis in each instance was verified at the biological laboratory of the Bureau of Science by a bacteriologic examination of the fæces. It seemed better, as was done by Rogers, to estimate the chlorides as an index of the most important changes in the salt content of the blood, rather than to determine the total ash, because the latter course never gives such exact results.

Twenty or 30 cubic centimeters of the blood were poured into a glass-stoppered weighing-bottle filled with a mixture of oxalate solution and a few drops of formalin, the weight of these fluids having previously been determined. To be used for the determination of the total solids, a second sample of 2 to 5 cubic centimeters was at the same time put into a weighed, empty, glass-stoppered bottle. The two were then sent to the laboratory as quickly as possible, again weighed, and weighed portions of the samples taken for analysis. The content of chlorides alone was determined in the first sample, and, as a rule, two parallel analyses were made.

It was my intention to make the first determinations according to Neuman's method[4] which in former experiments has given very satisfactory results.

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[2] *This Journal, Sec. B* (1909), **4**, 99.

[3] *Therap. Gaz.* (1909), **33**, 761.

[4] *Ztschr. physiol. Chem.* (1903), **37**, 115.

Not having the usual apparatus at hand, I improvised one, but the results were too low, either because the glass connection, ground by myself, was not absolutely tight, or the 1-liter bottle was too large. I therefore abandoned this method and mixed the blood with 2 or 3 grams of chemically pure sodium carbonate, dried it in a nickel dish and incinerated it at a very low temperature but only to the point when all organic material was destroyed, no effort being made to burn the charcoal. The incinerated material was extracted several times with hot water, the filtrate precipitated with silver nitrate, and the ash and filter then washed with dilute nitric acid. The extracts very often had a slightly yellow color. In these cases the following technique proved to be very satisfactory:

After precipitation with silver nitrate, the solution was boiled and a 5 per cent solution of potassium permanganate added, drop by drop, until a faint rose color appeared; the excess of potassium permanganate was then reduced by a few drops of ferro-ammonium-sulphate solution. The permanganate oxydized all the light yellow organic material, the fluid became clear, and at the same time the silver chloride became fully flocculent. The latter was determined gravimetrically in some cases, and one of the two samples was titrated by Volhard's method.

The water content of the blood was determined in the second sample by drying at 99° to 100° to a constant weight. Several samples of blood serum from cholera patients were also analyzed in the same way. Obviously, it is more difficult to secure a sufficient amount of serum than it is of blood, for analyses. For comparison, I analyzed blood and blood-serum of Filipino boys and students. I found practically the same composition as obtains with normal Europeans.

The second part of the study covered the examination of the blood of persons dead of cholera. While one would expect to find a pronounced change in the composition of the blood in cases which die and which therefore are the most severe, yet, so far as I could discover, no analyses heretofore have been made of the blood of cholera corpses. Through the courtesy of Dr. Vernon L. Andrews, of the department of pathology of the Philippine Medical School, I received blood samples from a number of Filipinos shortly after death, in whom the post-mortem findings and the bacteriologic examination of the fæces established the diagnosis of cholera. The blood was taken from the large veins of the heart before performing the autopsy and samples of from 200 to 250 cubic centimeters were secured without trouble. The results of my analyses are recorded in the following tables. I also append those of Schmidt and, for comparison, some recent ones of *normal blood*.

According to the analyses of Biernacki,[5] Erben,[6] and Dennstedt and Rumpf,[7] the following values can be regarded as the normal, average composition of human blood.

[5] *Ztschr. klin. Med.* (1894), **24**, 460.

[6] *Ibid.* (1900), **40**, 266–293.

[7] *Ztschr. physiol. Chem.* (1904), **41**, 42–54.

Composition of normal blood.

	Solids.	Chlorides.	Na_2O.	K_2O.	P_2O_5.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Biernacki, Erben, and Dennstedt	19.7 to 22.8	0.26 to 0.28	0.16 to 0.19	0.14 to 0.18	About 0.08
Aron:					
Normal Filipino blood	21.3	0.265			0.085
Blood of M. Rosario who recovered from cholera	19.9	0.264			

Analyses of cholera blood (Schmidt).

No.	Sick.	Died.	Solids.	Chlorides.	P_2O_5.	K_2O.	Na_2O.	Phosphate of—	
								Calcium.	Magnesium.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
VII	3 hours	18 hours	25.26	0.228	0.031	0.194	0.158	0.032	0.096
II	7 hours	Cured	23.91						
IV	9 hours	18 hours	25.47	0.222	0.075	0.203	0.150	0.046	0.047
VI	12 hours	10 hours	24.30	0.259	0.089	0.225	0.140	0.074	
V	18 hours	Cured	21.94	0.221	0.061	0.166	0.172		
III	36 hours	24 hours	23.90	0.195	0.081	0.184	0.111	0.086	
I	48 hours	3 days	21.38						
Normal			21.02	0.262	0.077	0.173	0.190	0.039	

Analyses of cholera serum (Schmidt).

	Solids.	Chlorides.
	Per cent.	Per cent.
VII	13.83	0.354
II	12.01	
VI	16.08	0.296
V	10.41	0.305
III	11.18	0.314
I	9.97	

Analyses of blood of cholera patients (Aron).

No.	Name.	Stage of disease at time specimen was taken.	Chlorides, per 1,000.	Water.	Solids.	Remarks.
				Per ct.	*Per ct.*	
1	Basil. Pagad	Collapse, second day	2.13	----	----	
2	Primit. Agui	do	2.21	----	----	
3	Mar. Ablao	Collapse; 8 hours sick; recovered.	----	77.1	22.9	
4	Alf. Catonj	do	----	73.8	26.2	
5	Mar. Rosario	Collapse, first day; recovered.	2.27	71.8	28.2	Blood analyzed after recovery as "normal" blood.
6	Juan Tobias	Collapse, second day; died	2.31	73.5	26.5	
7	Kuada Diose	End of collapse; third day died.	2.30	71.5	28.5	
8	Eulal. Gonzal	Beginning uræmia	2.17	78.5	21.5	
9	Herb. Mercado	Uræmia fifth day; recovered.	2.25	78.5	21.5	
10	Eug. Mendoza	Uræmia sixth day; died	1.94	77.6	22.4	
----	Maxim. Aorro	Died	2.45	----	----	Bacteriologically not cholera.

Analyses of serum of cholera patients (Aron).

Name.	Stage of disease.	Chlorides, per 1,000.	Water.	Solids.	Remarks.
			Per ct.	*Per ct.*	
Maximo Aorro	8 hours sick	3.44	87.6	12.4	Bacteriologically not cholera.
Maria Albao	8 hours sick; collapse	3.10	87.1	12.9	Died sixth day.
Ino. Villera	15 hours sick; collapse	3.15	85.8	14.2	Recovered.
Brig. Panjamban	Uræmia	2.58	87.7	12.3	Died seventh day.

Analyses of normal serum of Filipinos (Aron).

	Chlorides (per cent).	Water (per cent).	Solids (per cent).
Fi.	3.84	90.6	9.4
Ga.	3.64	90.4	9.6
Sa.	......	91.4	8.6

Analyses of cholera blood obtained at autopsy (Aron).

Name.	Solids.	Water.	Cholorides, per 1,000.	P_2O_5, per 1,000.	CaO, per 1,000.	K_2O, per 1,000.	Na_2O, per 1,000.	Protein N × 6¼.
	Per cent.	*Per cent.*						*Per cent.*
Dem. Esguerr	--------	--------	2.24	--------	--------	--------	--------	--------
A. del Ros	--------	--------	2.18	--------	--------	--------	--------	--------
Andr. Vent	27.6	72.4	--------	--------	--------	--------	--------	--------
Paul Romano	28.3	71.7	2.22	--------	--------	1.98	1.29	27.1
Euseb. Gonz	26.3	73.7	2.16	1.15	--------	--------	--------	25.9
Lor. Caccha	28.8	71.2	2.26	1.30	0.08	--------	--------	27.3
2 cases 1908	28.01 27.46	--------	--------	--------	--------	--------	--------	--------

All of the blood samples obtained at autopsy show a more or less decided decrease of water and a corresponding increase of solids. Taking about 20 per cent of solids as normal, the increase amounts to as much as 8.5 per cent.

The blood from cholera patients, in four cases, shows a decrease in the water content of the same degree as that found in the dead bodies, but four others had either a normal water content, or only a slight diminution. Therefore, it would be but natural to conclude that the blood is more highly concentrated in severe cases than in mild ones. Unfortunately, there is no accurate criterion of the severity of a given case, but if we assume that the patients who recover constitute the mild cases and those who die the severe ones, then there is not the slightest indication that the water content bears any relation to the severity of the disease. While the number of analyses I have made is too small to enable us to arrive at any final conclusion, I do not believe it will be possible to fix a constant relationship between the severity of the disease and the loss of water from the blood, because the interval of time during which the loss of water takes place must play an important rôle.

Another fact would seem to be of importance: The four cases with a low water content of the blood were in the early stages of the disease at the time the samples were taken, having been sick for from one to three days only. The figures for these cases agree well with the results obtained by Schmidt, for his patients also show an increase of solids in the blood in the early, but not in the later stages of cholera. The chloride content of the blood is decreased in nearly all instances investigated by myself as well as by Schmidt, a result which so far agrees fully with that of Rogers.

Is Rogers's conclusion that the blood has become hypotonic also correct? We have seen that the blood of cholera patients loses water. An *isotonic* blood, with a lower content of water, should have a lower content of salts (chlorides). The decrease in salts (chlorides) in itself does not prove that the blood has become *hypotonic*. The quantity of salts is often reduced in relation to the amount of total solids, but this calculation is apt to convey a wrong idea with regard to the tonicity of the fluid. The quantity of the salts should be compared in relation to the quantity of water and then it should be determined whether the loss in salts is proportional to the loss in water, or greater or less. If we do this, we will see that in the samples obtained during the first three days of the disease we can scarcely speak of a greater loss in the salts than would correspond to that of water. Of course, in the later stage of the disease, in Schmidt's analyses as well as in my own, the water content of the blood is almost normal, while that of salts, estimated as chlorides, is below that point. At this time, which corresponds to that of the uræmic stage of the disease, we really would have a hypotonic blood. It is worth noting that Rumpf[8] and Dennstedt also found a very great decrease in salts with but slight decrease in water in the blood of a man dying of nephritis (in uræmia).

The composition of the blood obtained at autopsy is exactly the same as that in the stage of collapse, during which all of these persons died. The water in almost all of these instances is decreased to 72 per cent. I found in this blood a marked decrease in the content of sodium, and a slight increase in that of potassium and of phosphorus. The results agree with those of the more extended analyses of Schmidt. This increase in potassium is readily explained if we consider that cholera blood contains a higher percentage of red blood cells than is normal. Schmidt pays special attention to the increase in phosphorus. While I found the same, I do not consider it at all remarkable, for the rise in the percentage of red blood cells and protein probably accounts for the higher phosphorus content of the blood.

The analyses of the sera also show a decrease of water and chlorides in cholera as compared with normal serum. The loss of the blood in water, as shown by these analyses as well as by those of Schmidt, is partly due to the loss of the serum in water, but the latter must also leave the red blood cells. The reduction of chlorides and water shown by the analyses of the serum confirms the observation made on samples of the entire blood.

SUMMARY.

In the stage of collapse in cholera a loss of water in the blood is regularly encountered, accompanied by a corresponding loss of chlorides (salts). This water loss is constantly high in the blood of persons who have died of cholera. In the later stages of the disease, the blood again shows an almost normal content of water, but the salts are not replaced to the normal amount. Therefore, the blood at this stage has a diminished salt content and is hypotonic.

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[8] *Münchener med. Wchnschr.* (1905), 52, 393.

CHOLERA AND CHOLERA-LIKE VIBRIOS ENCOUNTERED IN THE PHILIPPINES.

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This paper is presented with the idea of elucidating some questions which arose in connection with the routine examination for cholera vibrios in the stools from suspected cholera cases.

The primary questions in the study were: (1) Whether any "cholera-like" vibrios in the Philippines might agglutinate in a low dilution of a specific anticholera serum and thus lead to error in the ordinary routine agglutination tests performed for the purpose of ascertaining the presence of cholera vibrios in the stools of cholera suspects or "carriers;" (2) whether any cholera vibrios lose their morphology or agglutinability under any conditions, but especially in mixed cultures sent in from the provinces for diagnosis; (3) whether any vibrios of the *El Tor* type could be discovered in the Philippines. As the work progressed two other questions arose: (4) Whether any of the "cholera-like" vibrios which do not agglutinate with a specific anticholera serum can under any conditions be made to acquire such agglutinability; (5) whether Dieudonne's medium is satisfactory for the isolation of cholera vibrios from the stools, and if so, whether it can be used to differentiate "cholera-like" vibrios from true cholera vibrios. Finally, the question (6) as to whether the hog might act as a "cholera carrier" in the Philippines, was investigated.

The work is not completed, but owing to the departure of both of us from the Philippines it has seemed advisable to publish at the present time what has been accomplished in the study.

We selected for the work a series of vibrios isolated by various members of the staff and ourselves from specimens of fæces received at the laboratory during the summer and autumn of 1909 and first divided the organisms into two groups, cholera and noncholera vibrios, as differentiated by their reactions with a cholera serum of high potency.

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We have excluded from the "cholera-like" vibrios water vibrios which may be differentiated readily from the cholera organisms, and which have no right to be classified as "cholera-like" organisms; and retained in the class of noncholera vibrios those isolated from human intestinal contents or from water and which are indistinguishable from the cholera organisms except by the agglutination test, or by Pfeiffer's reaction.

TABLE I.—*Cholera and noncholera vibrios studied.*

CHOLERA VIBRIOS STUDIED.

No.	Name.	Date.	Origin.	Remarks
		1909.		
1	Regala I	May 27	Human intestine.	Typical cholera, Pampanga.
2	Regala II	June 10	do	Do.
3	Salas	June 19	do	Do.
4	Miraflores	June 25	do	Do.
5	Nicolas	June 20	do	Do.
6	David	June 21	do	Do.
7	Bautista	July 10	do	1 typical cholera; first case in Manila, 1909.
8	Ramon	July 6	do	Typical cholera, Albay.
9	Succro	July 9	do	Do.
10	C. Robles	Aug. 31	do	Typical cholera, Bulacan.
11	Dizon		do	Typical cholera, Manila.
12	Aniceto		do	Typical cholera, Pangasinan.
13	Maquio	Oct. 9	do	Typical cholera, Cavite.
14	Sucgang		do	Reported as infantile convulsions, Manila.
15	Calderon		do	Typical cholera, Manila.
16	Lorenzo		do	Do.
17	Cavite I		do	Do.
18	Cavite II		do	Do.
19	953		do	Do.
20	955		do	Do.
21	956		do	Do.
22	989		do	Do.
23	1029		do	Do.
24	1038		do	Do.
25	1041		do	Do.
26	1057		do	Do.
27	1101		do	Do.
28	1104		do	Do.
29	1108		do	Do.
30	1131		do	Do.
31	Camay	June 1	do	Typical cholera specimen received on agar after 15 days in transit from Mindanao.
32	Abalogan	July	do	Typical cholera specimen on agar, 7 days in transit from Samar.
33	Berezosa	July 7	do	Light case; specimen on agar 7 days in transit from Samar.
34	León	July 28	do	Typical case; dead in 5 hours, Manila.
35	Solitana		do	Typical case; specimen on agar 7 days in transit from Oriental Negros.

TABLE I.—*Cholera and noncholera vibrios studied*—Continued.

NONCHOLERA VIBRIOS STUDIED.

No.	Name.	Date.	Origin.	Remarks.
		1909.		
1	No. 135	Apr. 15	Human intestine	Amœbic dysentery.
2	Iris	June ?	Water	Estero at Calle Iris.
3	Notbil	June 6	Human intestine	Necropsy, pelvic peritonitis, salpingitis.
4	Gapus	Aug. 18	do	Post-mortem decomposition of all organs.
5	Sande	Sept. 8	do	Mild case of cholera. This vibrio found in addition to true cholera vibrios.
6	Sta. Monica	Sept. 11	Water	Stagnant pool which received human excretions.
7	Malabon	do	do	Specimen from water left in fishing boats after the fish had been unloaded.
8	Hagonoy	do	do	From water in bottom of boats after fish had been unloaded.
9	Oruni	Oct. ?	do	From fishing boats.
10	Navotas A	Oct. 14	Clams	
11	Navotas B	do	do	
12	Navotas C	do	do	
13	Sandejas A		Water	Stagnant water which received house wastes, including human excrement.
14	Sandejas B		do	
15	Sandejas C		do	
16	Sandejas D		do	
17	Carolina		do	Large unprotected well without walls.
18	Bilibid		do	"Estero de Bilibid" which receives human excrement.
19	869		Human intestine	
20	568		do	
21	371		do	
22	75		do	
23	48		do	
24	21		do	

Great difficulty was experienced formerly in securing bacteriological diagnoses of suspicious cases of cholera occurring in distant provinces. Often the examination of the specimens of fæces sent was futile. On the other hand, it was found that if ordinary alkaline agar slants were inoculated from the fæces and the cultures shipped in tin mailing cases cholera vibrios could frequently be isolated from such tubes even after they sometimes had been fifteen days in transit.

Two points should be accentuated in giving directions to health officers in distant places for the preparation of these cultures: (1) To place very little material on the slant; (2) to secure the specimen in the first twenty-four hours of the illness, if possible. When these directions are followed, the tubes often present isolated colonies of cholera vibrios upon arrival at the laboratory, and even if they do not appear so promising, recovery of the vibrios from them is often not difficult by the ordinary peptone and agar plate method.

We have found no media which would differentiate cholera from noncholera vibrios, although we have had an opportunity to test the elective blood-alkali-agar medium of Dieudonné (2) with fresh cholera material. *Cholera vibrios grow luxuriantly upon this medium, but other cholera-like vibrios also grow well.* However *Bacillus typhosus* does not develop on it and colon bacilli scarcely grow at all. Some other intestinal bacteria also flourish, but not luxuriantly, and less care is necessary in manipulation because many air-borne organisms either do not grow at all or develop so slowly as to be a negligible quantity. Tested side by side with ordinary agar plates, the relative number of cholera colonies is enormously increased, they develop faster, and, in favorable cases upon direct inoculation without preliminary enrichment in peptone, the diagnosis may be made therefrom in ten hours.[3]

Some of the noncholera vibrios which we examined did not differ more markedly from cholera vibrios in morphology, motility, cultural characteristics, production of cholera-red and pathogenicity, than may two individual cholera strains. They develop in peptone with the same rapidity and the colonies present the same appearance upon agar plates as do those of cholera vibrios.

Zlatogoroff, (12) in an interesting article, has called attention to the various characteristics of the cholera vibrio which have been relied upon for diagnosis and found wanting in the past, namely, growth upon gelatine, cholera-red production, absence of phosphorescence, absence of hæmolytic power, failure to produce a soluble toxin, and others. He admits the specificity of Pfeiffer's reaction, but is inclined to think that agglutinability is a quality which might be acquired and lost with surprising facility. He concluded that the life of cholera vibrios in water reduced their agglutinability, but that this quality could be restored by certain procedures.

Zlatogoroff worked with a total of 23 vibrios and a cholera serum with an agglutinating limit of 1–20,000. He found that 5 of these strains would agglutinate in a dilution of 1–5,000 and he at once considered them to be cholera vibrios. After one month he noticed that 3 of the others showed agglutination in a dilution of 1–500. These had been transplanted once a week and kept twenty-four hours in incubators at 37°C. and then at 16° to 18°C.

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[3] Doctor Bowman of the biological laboratory, Bureau of Science, has used this medium in the routine examination of 200 specimens sent by the Bureau of Health. The specimens were received as fæces, agar streak cultures, peptone cultures, portion of the intestine, etc. Streak cultures were made directly on the Dieudonné medium. When cholera was present the organism grew luxuriantly, overgrowing all others. A loop of the culture on the plates was transferred to a drop of agglutinating serum on a slide, and the diagnosis quickly made, while in using ordinary alkaline agar plates it was necessary sometimes to examine several colonies before a positive reaction was obtained. The use of this medium certainly simplifies the technique in the rapid bacteriologic diagnosis of cholera and at present it is being employed in the routine examination of all specimens sent to this laboratory.

He passed his organisms through animals (guinea pigs), once in three or four weeks, and after 54 generations raised the agglutinability so that it was exhibited in a dilution of 1–10,000. He was able to accomplish this only after he combined animal passage at rather infrequent intervals, with frequent transplanting upon good media. Neither of these methods alone sufficed to develop agglutinative properties. For example: Culture 15; dose ⅙ slant; November 6, no agglutination; November 6, ⅓ slant, guinea pig, died on November 9. Culture peritoneal on agar; November 10, agglutination 1–200; November 24, same repeated, agglutination 1–400; the same culture passed through 4 pigs without intermediate culture only developed an agglutinative power of 1-100.

He continued this treatment for three months, at the end of which time the vibrio had agglutinated in a dilution of 1–5,000. The culture transplanted without animal passage for three months agglutinated in 1–200.

To summarize his work, there were in all twenty-three vibrios studied: 5 agglutinated at the beginning in a dilution of 1–5,000; the agglutinating titre of the serum was 1–20,000; 18 vibrios did not agglutinate; 10 acquired agglutinative properties after six months, in a dilution of 1–5,000; 7 remained negative; and 1 died out.

Zlatogoroff concluded that during cholera epidemics, in addition to typical forms, atypical cholera vibrios which have lost certain biologic qualities may also be present in water. In time, under certain artificial conditions, these vibrios regain all the qualities of the typical cholera vibrios. He states: "Failure to agglutinate these water vibrios does not prove that they are not cholera, as cholera vibrios can change into saphropytic subvarieties very easily and can also lose their ability to agglutinate with cholera serum."

Barrenscheen (1) repeated that part of the work of Zlatogoroff which related to the reduction of agglutinability by keeping the vibrio for varying periods in water. He found that a cholera vibrio which agglutinated in a dilution of 1–40,000 at the beginning, did not agglutinate above 1–5,000 after eight days in distilled water. Cultures upon agar from the bacterial residue after centrifugation was also tested. The agglutinability had dropped to 1-400. Restoration or increase of agglutinability was not achieved after three weeks, although Barrenecheen succeeded in increasing the luxuriance of growth. He concludes that there is a passing out of the agglutinable substance into the water.

To test whether noncholera vibrios may acquire agglutinability to cholera serum, 12 of our own strains which seemed most favorable were selected. They all possessed a morphology, motility, and cholera-red production similar to those of cholera vibrios. They were treated in a manner similar to that suggested by Zlatogoroff. They were passed through guinea pigs between November 1 and 8, December and January, and transplanted upon agar every five or six days. After each animal passage, agglutination tests were made with a good cholera serum. At the end of two months, with 3 animal passages and 12 intermediate transplantations, not the slightest change was noticeable in the behavior of the organism to cholera agglutinating serum.

Our vibrios isolated from the human intestines, from water or other sources which are negative to agglutination tests with cholera serum and *classified by us as noncholera, do not develop agglutinability to cholera serum when treated as described by Zlatogoroff.*

The vibrios isolated by him from water and which "developed" agglutinability with cholera serum in a dilution of 1–10,000 must have been cholera vibrios.

For instance, his culture No. 15, cited above; with a dose of ⅛ slant, on November 6 showed no agglutination; ⅓ of a slant was injected into guinea pig. The guinea pig died on November 9. One transplant was made on November 10 and the agglutination was 1–200.

On the other hand, we have not been able at all to increase agglutinability.

Preparation of sera.—Sera were prepared with 10 cholera strains, 2 *El Tor* strains, and from 9 noncholera vibrios in the following manner: Eighteen-hour cultures on agar were used; suspensions were made with salt solution. Each rabbit received 4 or 5 injections; the interval between injections usually being seven to eight days. The amounts injected were as follows:

First day, 1 loop heated one hour at 60°C., into ear vein.
Eighth day, 3 loops heated one hour at 60°C., into ear vein.
Sixteenth day, 5 loops heated one hour at 60°C., into ear vein.
Twenty-fourth day, ½ slant, living, intraperitoneally.
Thirty-second to thirty-fourth day, 1 slant, living, intraperitoneally.
On the fortieth to forty-fourth day the animal was bled.

Selection of an animal for the preparation of a specific serum.—Kolle and Gotschlich (4) tested the normal sera of rabbit, ass, horse, goat and ox upon vibrios and found that normal rabbit and ass sera had a very slight agglutinating action upon vibrios, while horse and goat sera sometimes exert an agglutinating action in a dilution of 1–40 or 1–50. The small quantity of serum obtainable from a rabbit is usually considered a great disadvantage, but we found that large amounts of good serum could be obtained by the following procedure:

The bleeding apparatus consisted of an ordinary, large test tube with a rubber cork perforated to admit two glass tubes. A short piece of rubber tubing and a long slender needle is attached to one of these glass tubes; the other is connected with the vacuum pipe by about a meter of heavy rubber tubing. After sterilization, the needle is inserted below the ensiform cartilage and a trifle to the left, and then pushed upward into the pericardium and heart. After passing through the integument the needle in passing upward meets very slight resistance until the pulsating heart is encountered, but with a slight additional pressure it passes through the ventricular wall and blood begins to drip into the tube. The vacuum is turned on to facilitate the flow. In this way 10 to 15 cubic centimeters can be obtained without trouble at one bleeding.

Rabbits may be bled upon three successive days, the product of the three bleedings being combined and tested at the same time. In this way plenty of serum is available and the rabbits are apparently unharmed by the operation.

In 1895 the use of cholera agglutinin in the differential diagnosis between cholera and similar vibrios was recommended by Gruber and Durham (3) and at about the same time by Pfeiffer and Kolle (9).

The relation of nonspecific vibrios which resembled cholera, to the latter was clearly defined and the absolute specificity of the cholera vibrio demonstrated by the work of Kolle and Gotschlich (4).

They found that many vibrios existed which morphologically and biologically were indistinguishable from the real cholera organism, except by the use of a specific cholera serum. Such a specific cholera serum which agglutinated cholera organisms in a dilution of 1-5,000 or 1–10,000 had no more effect upon noncholera vibrios than normal serum from the same species of animal and sera prepared from noncholera vibrios had as little effect upon cholera vibrios.

Strong agglutinating cholera serum, in consequence of its richness in cholera agglutinin in dilutions of 1–2,000, 1–3,000, 1–5,000, serves for differentiating cholera from nonspecific vibrios. Kolle remarks that differences in the agglutinability of individual cholera strains make little difference, as the strains most refractory to this reaction will show positive results in an hour.

Kolle and his co-workers were unable to produce a serum with the noncholera vibrios which had any more effect upon genuine cholera vibrios than normal rabbit serum. He noted a great variation among the individual cholera strains in morphology, motility, cholera-red production, pathogenicity and cultural characteristics, and pointed out the unreliability of any or all of these characteristics and the absolute necessity of employing the agglutination test before making a diagnosis. With the exception of the old laboratory strains of Pfeiffer and Hankin, which seemed to be extremely susceptible to all agglutinating cholera sera, Kolle's cholera sera agglutinated the majority of cholera strains in a dilution 1–2,000 to 1–5,000, occasionally in higher dilutions, but in no instance did a cholera strain fail to agglutinate in a dilution of at least 1 to 1,000.

TABLE II.—*Agglutination tests.*

Name of culture.	Salt control.	Normal rabbit serum.			Serum Regala II.							
		1–10.	1–20.	1–50.	1–10.	1–20.	1–50.	1–100.	1–200.	1–500.	1–1,000.	1–2,000.
Regala I	—	—	------	------	+	+	+	+	+	+	+	+
Regala II		-	------	------	+	+	+	+	+	+	+	+
Salas	—	—	------	------	+	+	+	+	+	+	+	+
Miraflores	—	+	—	—	+	+	+	+	+	+	+	+
Nicolas	—	—	------	------	+	+	+	+	+	+	+	+
David	—	+	—	—	+	+	+	+	+	+	+	+
Bautista	—	------	------	------	+	+	+	+	+	+	+	
Ramon	—	—	—	—	+	+	+	+	+		+	+
Sucero	—	—	------	------	+	+	+	+	+		+	
C. Robles	—		------	------	+	+	+	+	+	+	+	+
Dizon	—	—	------	------	+	+	+	+	+	+	+	+
Anicete		—	------	------	+	+	+	+	+	+	+	+
Maquio		—	------	------	+	+	+	+		+	+	+

TABLE II.—*Agglutination tests*—Continued.

Name of culture.	Salt control.	Normal rabbit serum.			Serum Regala II.							
		1–10.	1–20.	1–50.	1–10.	1–20.	1–50.	1–100.	1–200.	1–500.	1–1,000.	1–2,000.
Sucgang	—	—			+	+	+	+	+	+	+	+
Calderon	—	—			+	+	+	+	+	+	+	+
Lorenzo S.	—	—			+	+	+	+	+	+	+	+
Cavite I	—	—			+	+	+	+	+	+	+	+
Cavite II	—	—			+	+	+	+	+	+	+	+
953	—	—			+	+	+	+	+	+	+	+
955	—	—			+	+	+	+	+	+	+	+
956	—	—			+	+	+	+	+	+	+	+
989	—	—			+	+	+	+	+	+	+	+
1029	—	—			+	+	+	+	+	+	+	+
1038	—	—			+	+	+	+	+	+	+	+
1041	—				+	+	+	+	+	+	+	+
1057	—	—			+	+	+	+	+	+	+	+
1101	—	—			+	+	+	+	+	+	+	+
1104	—	—			+	+	+	+	+	+	+	+
1108	—	—			+	+	+	+	+	+	+	+
1131	—	—			+	+	+	+	+	+	+	+
Camay	—	—			+	+	+	+	+	—	—	—
Abologan	—	—			+	+	+	+	±	—	—	
Berezosa	—	—			+	+	+	+	±	—	—	
Leon	—	—			+	+	+	+	±	—	—	
Soletana	—	—			+	+	+	+	±	—	—	
135	—	+	—		+	—	—					
Gapus					—	—	—					
Notbil	—	+	+		+	—	—					
Sande	—	+	±		+	—	—					
Santa Monica	—	—	—		—	—	—					
Malabon	—	+	—		—	—	—					
Hagonoy	—	+	—		—	—	—					
Orani	—				—	—	—					
Navotas A	—	—			—	—	—					
Navotas B	—	—			—	—	—					
Navotas C	—	—			—	—	—					
Sandejas A	—	—	—		+	—	—					
Sandejas B	—	—			—	—	—					
Sandejas C	—	—			—	—	—					
Sandejas D	—	—			—	—	—					
Carolina	—	—	—		—	—	—					
Bilibid	—	—			—	—	—					
869	—	—			—	—	—					
568	—	—			—	—	—					
371	—	—			—	—	—					
75	—	—			—	—	—					
48	—	—			—	—	—					
21	—	—			—	—	—					
Iris	—	—			—	—	—					

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum B. Robles.											
	1–10.	1–20.	1–50.	1–100.	1–200.	1–300.	1–400.	1–500.	1–1,000.	1–2,000.	1–4,000.	1–8,000.
Abalogan	+	+	+	+	+	+	+	+	+	—		
Berezosa	+	+	+	+	+	+	+	+	±	—		
Leon	+	+	+	+	+	+	+	+	±	—		
Solitana	+	+	+	+	+	+	+	+	±	—		
Ramon	+	+	+	+	+	+	+	+	+	+	+	+
Sucero	+	+	+	+	+	+	+	+	+	+	+	+
C. Robles	+	+	+	+	+	+	+	+	+	+	+	±
Dizon	+	+	+	+	+	+	+	+	+	+	+	+
Anicete	+	+	+	+	+	+	+	+	+	+	+	±
Maquio	+	+	+	+	+	+	+	+	+	+	+	±
Sucgang	+	+	+	+	+	+	+	+	+	+	+	±
Calderon	+	+	+	+	+	+	+	+	+	+	+	±
Iris	—											
Notbil	—											
Sta. Monica	—											
Sande	—											
Malabon	+	+	+	—								
Hagonoy	—											
Orani	—											
Navotas A	—											
Navotas B	—											
Sandejas A		±	—	—								
Sandejas B		tr.	—	—								
Gapus	—											
Bilibid	—											
B. Robles	+	+	+	+	+	+	+	+	+	+	+	±
King A	+	+	+	+	+	+	+	+	+	+	+	±
King B	+	+	+	+	+	+	+	+	+	+	+	±
869	—											
568	—											
371	—											
75	—											
48	+	tr.	—	—								
21	—	—										

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum King A.										
	1-10.	1-20.	1-50.	1-100.	1-200.	1-300.	1-400.	1-500.	1-1,000.	1-2,000.	1-4,000.
Abalogan	+	+	+	+	±	+	+	±	—	—	-----
Berezona	+	+	+	+	+	+	+	±	—	—	-----
Leon	+	+	+	+	+	±	+	±	—	—	-----
Soletana	+	+	+	+	±	+	+	±	—	—	-----
Ramon	+	+	+	+	+	+	+	+	+	+	±
Sucero	+	+	+	+	+	+	+	+	+	+	±
C. Robles	+	+	+	+	+	+	+	+	+	+	±
Dizon	+	+	+	+	+	+	+	+	+	+	±
Aniecte	+	+	+	+	+	+	+	+	+	+	±
Maquio	+	+	+	+	+	+	+	+	+	+	±
Suegana	+	+	+	+	+	+	+	+	+	+	+
Calderon	+	+	+	+	+	+	+	+	+	+	+
Iris	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Notbil	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Sta. Monica	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Sande	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Malabon	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Hagonoy	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Orani	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Navotas A	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Navotas B	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Sandejas A	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Sandejas B	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Bilibid	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
Gapus	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
King A	+	+	+	+	+	+	+	+	+	+	+
King B	+	+	+	+	+	+	+	+	+	+	±
869	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
568	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
871	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
75	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
48	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----
21	—	—	—	-----	-----	-----	-----	-----	-----	-----	-----

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum Abalogan.								
	1-10.	1-20.	1-50.	1-100.	1-200.	1-500.	1-1,000.	1-2,000.	1-4,000.
Abalogan	+	+	+	+	+	+	+	±	------
Berezosa	+	+	+	+	+	+	+	±	------
Leon	+	+	+	+	+	+	+	±	------
Soletana	+	+	+	+	+	+	+	±	------
Ramon	+	+	+	+	+	+	+	±	------
Lucero	+	+	+	+	+	+	±	—	------
C. Robles	+	+	+	+	+	+	±	—	------
Dizon	—	+	+	+	+	+	±	—	------
Aniecte	+	+	+	+	+	+	±	—	------
Maquio	+	+	+	+	+	+	±	—	------
Sucgana	+	+	+	+	+	+	+	±	------
Calderon	+	+	+	+	+	+	+	±	------
135	+	+	+	+	+	------	------	------	------
Iris	—	—	------	------	------	------	------	------	------
Notbil	tr.	—	------	------	------	------	------	------	------
Sta. Monica	tr.	—	------	------	------	------	------	------	------
Sande	tr.	—	------	------	------	------	------	------	------
Malabon	+	±	—	------	------	------	------	------	------
Hagonoy	±	—	—	------	------	------	------	------	------
Navotas A	+	±	—	------	------	------	------	------	------
Navotas B	±	—	------	------	------	------	------	------	------
Sandejas A	+	+	±	—	—	------	------	------	------
Sandejas B	+	+	±	—	—	------	------	------	------
Bilibid	—	—	—	------	------	------	------	------	------
Gapus	—	—	—	------	------	------	------	------	------
Orani	—	—	—	------	------	------	------	------	------
King A	+	+	+	+	+	+	+	+	—
King B	+	+	+	+	+	+	+	+	—
869	—	—	—	------	------	------	------	------	------
568	—	—	—	------	------	------	------	------	------
371	—	—	—	------	------	------	------	------	------
75	—	—	—	------	------	------	------	------	------
48	—	—	—	------	------	------	------	------	------
21	—	—	—	------	------	------	------	------	------
Carolina	—	—	—	------	------	------	------	------	------

TABLE II.—*Agglutination tests*—Continued.

Culture.	Salt.	Normal serum.		Serum Berezosa.								
		1-10.	1-20.	1-10.	1-20.	1-50.	1-100.	1-200.	1-400.	1-500.	1-1,000.	1-2,000.
Abalogan	—	—	----	+	+	+	+	+	+	+	+	+
Berezosa	—	—	----	+	+	+	+	+	+	+	+	±
Leon	—	—	----	+	+	+	+	+	+	+	+	±
Soletane	—	—	----	+	+	+	+	+	+	+	+	—
Ramon	—	—	----	+	+	+	+	+	+	+	+	----
Lucero	—	—	----	----	----	----	+	+	+	+	+	----
C. Robles	—	—	----	----	----	----	+	+	+	+	+	----
Dizon	—	—	----	----	----	----	+	+	—	+	+	----
Anicete	—	—	----	----	----	----	+	+	—	+	+	----
Maquio	—	—	----	----	----	----	+	+	—	+	+	----
Sucgana	—	—	----	----	----	----	+	+	—	+	+	----
Calderon	—	—	----	----	----	----	+	+	+	+	+	----
135	—	+	—	—	—	—	—	----	----	----	----	----
Iris	—	—	----	—	—	—	----	----	----	----	----	----
Notbil	—	—	----	—	—	—	----	----	----	----	----	----
Sta. Monica	—	—	----	—	—	—	----	----	----	----	----	----
Sande	—	—	----	—	—	—	----	----	----	----	----	----
Malabon	—	+	—	+	+	+	—	----	----	----	----	----
Hagonoy	—	—	----	—	—	----	----	----	----	----	----	----
Navotas A	—	—	----	—	—	----	----	----	----	----	----	----
Navotas B	—	—	----	—	—	----	----	----	----	----	----	----
Sandejas A	—	—	----	+	±	----	----	----	----	----	----	----
Sandejas B	—	—	----	+	±	----	----	----	----	----	----	----
Bilibid	—	—	----	—	—	----	----	----	----	----	----	----
Carolina	—	—	----	—	----	----	----	----	----	----	----	----
King A	—	—	----	+	+	+	+	+	+	+	+	----
King B	—	—	----	+	+	+	+	+	+	+	+	----
869	—	—	----	—	—	----	----	----	----	----	----	----
568	—	—	----	—	—	----	----	----	----	----	----	----
371	—	—	----	—	—	----	----	----	----	----	----	----
75	—	—	----	—	—	----	----	----	----	----	----	----
48	—	—	----	—	—	----	----	----	----	----	----	----
21	—	—	----	—	—	----	----	----	----	----	----	----
Gapus	—	—	----	—	—	----	----	----	----	----	----	----
Orani	—	—	----	—	—	----	----	----	----	----	----	----

TABLE II.—*Agglutination tests*—Continued.

Culture.	Salt.	Normal serum.			Serum Leon.								
		1-10.	1-20.	1-50.	1-10.	1-20.	1-50.	1-100.	1-200.	1-400.	1-500.	1-1,000.	1-2,000.
Abalogan	—	—	------	------	+	+	+	+	+	+	+	+	±
Berezosa	—	—	------	------	+	+	+	+	+	+	+	+	±
Leon	—	—	------	------	+	+	+	+	+	+	+	—	±
Soletana	—	—	------	------	+	+	+	+	+	+	+	+	±
Ramon	—	—	------	------	+	+	+	+	+	+	+	+	±
Lucero	—	—	------	------	—	+	+	+	+	+	+	+	+
C. Robles	—	—	------	------	—	+	+	+	+	+	+	+	±
Dizon	—	—	------	------	—	+	+	+	+	+	+	+	±
Anicete		—	------	------	—	+	+	+	+	+	+	+	------
Maquio	—	—	------	------	—	+	+	+	+	+	+	+	------
Suegana	—	—	------	------	+	+	+	+	+	+	+	+	∓
Calderon	—	—	------	------	+	+	+	+	+	+	+	+	+
135	—	—	------	------		—	—	------	------	------	------	------	------
Iris	—	—	------	------	—	—	—	------	------	------	------	------	------
Notbil	—	—	—	—	±	—	—	------	------	------	------	------	------
Sta. Monica	—	—	------	------	—	—	—	------	------	------	------	------	------
Sande		—	------	------	±	—	—	------	------	------	------	------	------
Malabon	—	±	—	—	±	±	—	------	------	------	------	------	------
Hagonoy	—	—	—	------	—	—	—	------	------	------	------	------	------
Orani	—	—	------	------	—	—	—	------	------	------	------	------	------
Navotas A	—	—	------	------	—		—	------	------	------	------	------	------
Navotas B	—	—	------	------	—	—	—	------	------	------	------	------	------
Gapus	—	—	------	------	—	—	—	------	------	------	------	------	------
Sandejas A	—	—	------	------	±	±	—	—	------	------	------	------	------
Sandejas B	—	—	------	------	±	—	—	------	------	------	------	------	------
Carolina	—	—	------	------	—	—	—	------	------	------	------	------	------
Bilibid	—	—	------	------	—	—	—	------	------	------	------	------	------
King A	—	—	------	------	+	+	+	+	+	+	—	+	±
King B	—	—	------	------	+	+	+	+	+	+	—	+	±
B. Robles	------	------	------	------	------	------	------	------	------	------	------	------	------
869	—	—	------	------	—	—	—	------	------	------	------	------	------
568	—	—	------	------	—	—	—	------	------	------	------	------	------
371	—	—	------	------	—	—	—	------	------	------	------	------	------
75	—	—	------	------	—	—	—	------	------	------	------	------	------
48	------	------	------	------	------	------	------	------	------	------	------	------	------
21	—	—	------	------	—	—	—	------	------	------	------	------	------
Regala II	—	—	------	------	+	+	+	+	+	+	+	+	±

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum Soletana.										
	1-10.	1-20.	1-50.	1-100.	1-200.	1-300.	1-400.	1-500.	1-800.	1-1,000.	1-2,000.
Abulogan	+	+	+	+	+	+	+	+	±	−	------
Berezosa	+	+	+	+	+	+	+	+	+	−	------
Leon	+	+	+	+	+	−	+	+	+	+	−
Soletana	+	+	+	+	+	+	+	+	+	−	−
Ramon	+	+	+	+	+	+	+	+	+	+	
Lucero	+	+	+	+	+	+	+	+	+	±	−
C. Robles	+	+	+	+	+	+	+	+	+	+	−
Dizon	+	+	+	+	+	+	+	+	+	−	−
Anicete	+	+	+	+	+	+	+	+	+	±	−
Maquio	+	+	+	+	+	+	+	+	±	−	−
Sucgana	+	+	+	+	+	+	+	+	+	+	−
Calderon	+	+	+	+	+	+	+	+	+	±	−
135	−	−	−	−	------	------	------	------	------	------	------
Iris	−	−	------	------	------	------	------	------	------	------	------
Notbil	−	−	------	------	------	------	------	------	------	------	------
Sta. Monica	−	−	------	------	------	------	------	------	------	------	------
Sande	−	−	------	------	------	------	------	------	------	------	------
Malabon	+	+	±	−	------	------	------	------	------	------	------
Hagonoy	−	−	------	------	------	------	------	------	------	------	------
Orani	−	−	------	------	------	------	------	------	------	------	------
Navotas A	−	−	------	------	------	------	------	------	------	------	------
Navotas B	−	−	------	------	------	------	------	------	------	------	------
Sandejas A	+	−	−	------	------	------	------	------	------	------	------
Sandejas B	+	−	−	------	------	------	------	------	------	------	------
Bilibid	−	−	------	------	------	------	------	------	------	------	------
Gapus	−	−	------	------	------	------	------	------	------	------	------
Carolina	−	−	------	------	------	------	------	------	------	------	------
King A	+	+	+	+	+	+	+	+	+	±	------
King B	+	+	+	+	+	+	+	+	+	------	------
869	−	−	------	------	------	------	------	------	------	------	------
568	−	−	------	------	------	------	------	------	------	------	------
371	−	−	------	------	------	------	------	------	------	------	------
75	−	−	------	------	------	------	------	------	------	------	------
48	−	−	------	------	------	------	------	------	------	------	------
21	−	−	------	------	------	------	------	------	------	------	------

TABLE II.—*Agglutination tests*—Continued.

Culture.	Salt solution.	Normal rabbit serum, 1–10.	Serum King B.						
			1–40.	1–80.	1–100.	1–500.	1–1,000.	1–1,500.	1–2,000.
Abalogan	−	−	+	+	+	+	+	±	−
Berezosa	−	−	+	+	+	+	+	±	−
Leon	−	−	+	+	+	+	+	±	−
Soletane	−	−	+	+	+	+	+	±	−
Ramon	−	−	+	+	+	+	+	±	−
Sucero	−	−	+	+	+	+	+	±	−
G. Robles	−	−	+	+	+	+	+	±	−
Dizon	−	−	+	+	+	+	+	±	−
Aniceta	−	−	+	+	+	+	±	−	−
Maquio	−	−	+	+	+	+	+	−	−
Sucgang	−	−	+	+	+	+	+	±	−
Calderon	−	−	+	+	+	+	+	±	−
King A	−	−	+	+	+	+	−	±	−
King B	−	−	+	+	+	+	+	+	−
869			−						
568			−						
75			−						
48			−						
371			−						
Iris			−						
Notbil			−						
Sta. Monica			−						
Sande			−						
Sandejas A			−						
Sandejas B			−						
Gapus			−						

Culture.	Salt solution.	Normal rabbit serum 1–10.	Serum No. 2.					
			1–400.	1–500.	1–800.	1–1,000.	1–1,500.	1–2,000.
Abalogan	−	−	+	+	+	+	+	±
Berezosa	−	−	+	+	+	+	+	tr.
Leon	−	−	+	+	+	+	+	±
Solitana	−	−	+	+	+	+	tr.	−
2	−	−	+	+	+	+	+	±
4	−	−	+	+	+	+	+	±
5	−	−	+	+	+	+	+	±
6	−	−	+	+	+	+	+	±

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum No. 4.					
	1-400.	1-800.	1-1,000.	1-1,500.	1-2,000.	1-4,000.
Abalogan	+	+	+	+	±	−
Berezosa	+	+	−	+	+	−
Leon	+	+	+	+	±	tr
Solitana	+	+	−	±	−	−
2	+	+	+	+	+	±
4	+	+	+	−	+	±
5	−	+	+		+	±
6	+	+	+	+	+	±

Culture.	Salt solution.	Normal rabbit serum, 1-10.	Serum No. 5.			
			1-500.	1-1,000.	1-1,500.	1-2,000.
Abalogan	−	−	+	+	±	−
Berezosa		−	+	+	±	−
Leon	−	−	+	+	±	−
Solitana	−	−	+	tr.	−	−
2	−	−	+	+	±	−
4	−	−	+	+	+	−
5	−	−	+	+	±	−
6	−	−	+	+	±	−

Culture.	Serum No. 6.					
	1-400.	1-800.	1-1,000.	1-1,200.	1-1,600.	1-2,000.
Abalogan	+	+	tr.	−	−	−
Berezosa	+	−	tr.	−	−	−
Leon	+	±	−	−	−	−
Solitana	+	+	tr.	−	−	−
2	+	+	+	+	±	tr.
4	+	+	+	+	±	tr.
5	+	+	+	+	±	tr.
6	+	+	+	+	±	tr.

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum Sandejas A.									
	1–10.	1–20.	1–50.	1–100.	1–200.	1–500.	1–1,000.	1–2,000.	1–4,000.	1–8,000.
Abalogan	—	—		------	------	------	------	------	------	------
Berezosa	—	—	—	------	------	------	------	------	------	------
Leon	—	—	—	------	------	------	------	------	------	------
Solitana	—	—	—	------	------	------	------	------	------	------
Ramon	+	—	—	------	------	------	------	------	------	------
Lucero	tr.	—	—	------	------	------	------	------	------	------
C. Robles	tr.	—	—	------	------	------	------	------	------	------
Dizon	tr.	—	—	------	------	------	------	------	------	------
Anicete	tr.	—	—	------	------	------	------	------	------	------
Maquio	+	—	—	------	------	------	------	------	------	------
Sucgana	tr.	—	—	------	------	------	------	------	------	------
Calderon	+	—	—	------	------	------	------	------	------	------
185	—	—	—	------	------	------	------	------	------	------
Iris	+	—	—	------	------	------	------	------	------	------
Notbil	+	—	—	------	------	------	------	------	------	------
Sta. Monica	—	—	—	------	------	------	------	------	------	------
Sande	—	—	—	------	------	------	------	------	------	------
Malabon	+	—	—	------	------	------	------	------	------	------
Hagonoy	—	—	—	------	------	------	------	------	------	------
Orani	—	—	—	------	------	------	------	------	------	------
Gapus	—	—	—	------	------	------	------	------	------	------
Navotas A	—	—	—	------	------	------	------	------	------	------
Navotas B	—	—	—	------	------	------	------	------	------	------
Sandejas A	+	+	+	+	+	+	+	+	+	±
Sandejas B	+	+	+	+	+	+	+	+	+	±
Carolina	—	—	—	------	------	------	------	------	------	------
Bilibid	—	—	—	------	------	------	------	------	------	------
B. Robles	—	—	—	------	------	------	------	------	------	------
King A	—	—	—	------	------	------	------	------	------	------
King B	—	—	—	------	------	------	------	------	------	------
869	—	—	—	------	------	------	------	------	------	------
568	—	—	—	------	------	------	------	------	------	------
371	—	—	—	------	------	------	------	------	------	------
75	—	—	—	------	------	------	------	------	------	------
48	+	+	+	+	+	+	+	+	+	±
21	—	—	—	------	------	------	------	------	------	------

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum Sandejas B.					
	1-50.	1-100.	1-200.	1-500.	1-1,000.	1-2,000.
Abalogan	—	—				
Berezosa	—	—				
Leon	tr	—				
Solitana	—	—				
Ramon	tr	—				
Lucero	tr	—				
C. Robles	tr	—				
Dizon	tr	—				
Aniecte	tr	—				
Maquio	tr	—				
Sucgana	tr	—				
Calderon	tr	—				
135	tr	—				
Iris	—	—	—			
Notbil	—	—				
Sande	—	—				
Sta. Monica	tr	—				
Malabon	tr	—				
Hagonoy	—	—	—			
Orani	—	—	—			
Nabotas A	—	—	—			
Nabotas B	—	—	—			
Gapus	—	—	—			
Sandejas A	+	+	—	+	+	±
Sandejas B	+	+	+	+	+	±
Carolina	—	—	—			
Bilibid	—	—	—			
King A	—	—	—			
King B	—	—	—			
B. Robles	—	—	—			
869	—	—	—			
568	—	—	—			
371	—	—	—			
75	—	—	—			
48	+	+	+	+	+	±
21	—	—	—			

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum Navotas B.							
	1–20.	1–50.	1–100.	1–200.	1–500.	1–1,000.	1–2,000.	1–4,000.
Abalogan	—	—	—	------	------	------	------	------
Berezosa	—	—	—	------	------	------	------	------
Leon	±	—	—	------	------	------	------	------
Solitana	—	—	—	------	------	------	------	------
Ramon	±	—	—	------	------	------	------	------
Lucero	±	—	—	------	------	------	------	------
C. Robles	±	—	—	------	------	------	------	------
Dizon	tr	—	—	------	------	------	------	------
Aniecte	tr	—	—	------	------	------	------	------
Maquio	tr	—	—	------	------	------	------	------
Suegana	±	—	—	------	------	------	------	------
Calderon	tr	—	—	------	------	------	------	------
135	—	—	—	------	------	------	------	------
Iris	—	—	—	------	------	------	------	------
Notbil	—	—	—	------	------	------	------	------
Sta. Monica	—	—	—	------	------	------	------	------
Sande	—	—	—	------	------	------	------	------
Malabon	+	+	—	------	------	------	------	------
Hagonoy	—	—	—	------	------	------	------	------
Orani	—	—	—	------	------	------	------	------
Navotas A	+	+	+	+	+	+	+	±
Navotas B	+	+	+	+	+	+	+	±
Gapus	—	—	—	------	------	------	------	------
Sandejas A	+	±	—	—	------	------	------	------
Sandejas B	+	+	tr	—	------	------	------	------
Carolina	—	—	—	------	------	------	------	------
Bilibid	—	—	—	------	------	------	------	------
King A	±	—	—	------	------	------	------	------
King B	—	—	—	------	------	------	------	------
B. Robles	—	—	—	------	------	------	------	------
869	—	—	—	------	------	------	------	------
568	—	—	—	------	------	------	------	------
371	—	—	—	------	------	------	------	------
75	—	—	—	------	------	------	------	------
48	+	—	—	------	------	------	------	------
21	—	—	—	------	------	------	------	------

TABLE II.—*Agglutination tests*—Continued.

Culture.	Salt solution.	Normal rabbit serum, 1-10.	Serum No. 869.					
			1-50.	1-100.	1-200.	1-500.	1-1,000.	
Abalogan	—	—	—	—	------	------	------	------
Berezosa	—	—	—	—	------	------	------	------
Leon	—	—	—	—	------	------	------	------
Soletana	—	—	—	—	------	------	------	------
Ramon	—	—	—	—	------	------	------	------
Succro	—	—	—	—	------	------	------	------
G. Robles	—	—	—	—	------	------	------	------
Dizon	—	—	—	—	------	------	------	------
Anicete	—	—	—	—	------	------	------	------
Maquio	—	—	—	—	------	------	------	------
Suegang	—	—	—	—	------	------	------	------
Calderon	—	—	—	—	------	------	------	------
King A	—			—	------	------	------	------
King B	—	—	—	—	------	------	------	------
869	—	—	+	+	+	+	+	+
568	------	------	—	—	------	------	------	------
75	------	------	—	—	------	------	------	------
48	------	------	—	—	------	------	------	------
371	------	------	—	—	------	------	------	------
Iris	------	------	—	—	------	------	------	------
Notbil	------	------	—	—	------	------	------	------
Sta. Monica	------	------	—	—	------	------	------	------
Sande	------	------	—	—	------	------	------	------
Sandejas A	------	------	—	—	------	------	------	------
Sandejas B	------	------	—	—	------	------	------	------
Gapus	------	------	—	—	------	------	------	------

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum No. 568.								
	1-20.	1-50.	1-100.	1-200.	1-500.	1-1,000.	1-2,000.	1-4,000.	1-8,000.
Abalogan	—	—	—						
Berezosa	—	—	—						
Leon	—	—	—						
Solitana	—	—	—						
Ramon	—	—	—						
Lucero	—	—	—						
C. Robles	—	—	—						
Dizon	—	—	—						
Anicete	—	—	—						
Maquio	—	—	—						
Sucgana	—	—	—						
Calderon	—	—	—						
135	—	—	—						
Iris	—	—	—						
Notbil	—	—	—						
Sta. Monica	—	—	—						
Sande	—	—	—						
Malabon	+	±	—						
Hagonoy	+	—	—						
Navotas A	tr	—	—						
Navotas B	—	—	—						
Sandejas A	+	—	—						
Sandejas B	+	—	—						
Bilibid	—	—	—						
Gapus	+	+	+	+	+	+	+	+	±
Orani	—	—	—						
B. Robles									
King A	+	±	—	—					
King B	+	±	—	—					
869	—	—	—						
568	+	+	+	+	+	+	+	+	—
371	—	—	—						
76	—	—	—						
48	+	—	—						
21	—	—	—						

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum No. 48.						
	1-50.	1-100.	1-200.	1-500.	1-1,000.	1-2,000.	1-4,000.
Abalogan	—	—	------	------	------	------	------
Berezosa	—	—	------	------	------	------	------
Leon	------	—	------	------	------	------	------
Solitana	—	—	------	------	------	------	------
Ramon	------	—	------	------	------	------	------
Sucero	------	—	------	------	------	------	------
C. Robles	------	—	------	------	------	------	------
Dizon	------	—	------	------	------	------	------
Anicete	------	—	------	------	------	------	------
Maquio	------	—	------	------	------	------	------
Sucgang	------	—	------	------	------	------	------
Calderon	------	—	------	------	------	------	------
135	------	—	------	------	------	------	------
Iris	—	—	------	------	------	------	------
Notbil	—	—	------	------	------	------	------
Sande	—	—	------	------	------	------	------
Sta. Monica	------	—	------	------	------	------	------
Hagonoy	—	—	—	------	------	------	------
Orani	—	—	—	------	------	------	------
Navotas A	—	—	—	------	------	------	------
Navotas B	—	—	—	------	------	------	------
Gapus	—	—	—	------	------	------	------
Sandejas A	+	+	+	+	+	+	±
Sandejas B	+	+	+	+	+	+	±
Carolina	—	—	—	------	------	------	------
Bilibid	—	—	—	------	------	------	------
King A	—	—	—	------	------	------	------
King B	—	—	—	------	------	------	------
B. Robles	—	—	—	------	------	------	------
869	—	—	—	------	------	------	------
568	—	—	—	------	------	------	------
371	—	—	—	------	------	------	------
75	—	—	—	------	------	------	------
48	+	+	+	+	+	+	±
21	—	—	—	------	------	------	------

Table II.—*Agglutination tests*—Continued.

Culture.	Serum Orani.							
	1-10.	1-20.	1-100.	1-500.	1-1,000.	1-2,000.	1-4,000.	1-8,000.
Abalogan	—	—						
Berezosa	—	—						
Leon	—	—						
Solitana	—	—						
Ramon	—	—						
Lucero	—	—						
C. Robles	—	—						
Dizon	—	—						
Anicete	—	—						
Maquio	—	—						
Sucgana	—	—						
Calderon	—	—						
135	—	—						
Iris	—	—						
Notbil	—	—						
Sta. Monica	—	—						
Sande	—	—						
Malabon	+	—						
Hagonoy	—	—						
Orani	+	+	+	+	+	+	+	+
Navotas A	—	—						
Navotas B	—	—						
Sandejas A	—	—						
Sandejas B	—	—						
Bilibid	—	—						
Gapus	—	—						
B. Robles	—	—						
King A	—	—						
King B	—	—						
869	—	—						
568	—	—						
371	—	—						
76	—	—						
48	—	—						
21	—	—						

TABLE II.—*Agglutination tests*—Continued.

Culture.	Serum Notbil.					
	1-20.	1-50.	1-100.	1-200.	1-500.	1-1,000.
Abalogan	—	—				
Berzosa	—	—				
Leon	—	—				
Solitana	—	—				
Ratuon	—	—				
Lucero	—	—				
C. Robles	—	—				
Dizon	—	—				
Aniecte	—	—				
Maquio	—	—				
Sucgana	—	—				
Calderon	—	—				
135	+	—				
Iris	+	—	—			
Notbil	+	+	+	+	+	±
Sta. Monica	+	+	+	+	—	—
Sande	+	+	+	+	+	±
Malabon	+	+	—			
Hagonoy	±	—	—			
Orani	—	—				
Navotas A	—	—				
Navotas B	—	—				
Sandejas A	—	—				
Sandejas B	—	—				
Gapus	—	—				
Bilibid	—	—	—			
B. Robles	+	—	—			
King A	—	—	—			
King B						
869	—	—				
568	—	—				
371	—	—				
75	+	+	+	—		
48	—	—				
21	—	—				

TABLE II. *Agglutination tests*—Continued.

Culture.	Normal serum, 1-10.	Serum Santa Monica.							
		1-20.	1-50.	1-100.	1-200.	1-300.	1-400.	1-500.	1-1,000.
Abalogan	—	-----	-----	—		—	—	—	-----
Berezosa	—	-----	-----	-----	-----	—	—	—	-----
Leon	—	-----	-----	-----	-----	—	—	—	-----
Soletana	—	-----	-----	-----	—	—	—	—	-----
Ramon	—	-----	-----	-----	-----	—	—	—	-----
Sucero	—	-----	-----	-----	-----	—	—	—	-----
G. Robles	—	-----	-----	-----	-----	—	—	—	-----
Dizon	—	-----	-----	-----	-----	—	—	—	-----
Anicete	—	-----	-----	-----	-----	-----	—	—	-----
Ma uio	—	-----	-----	-----	-----	-----	—	—	-----
Sucgang	—	-----	-----	-----	-----	-----	—	—	-----
Calderon	—	-----	-----	-----	-----	-----	—	—	-----
135	—	-----	-----	—	-----	-----	—	—	-----
Iris	—	—	—	—	—	-----	—	—	—
Notbil	±	+	+	+	+	±	+	—	—
Sande	—	+	+	+	+	-----	—	+	+
Sta. Monica	—	+	+	+	+	+	+	·	·
Malabon	+	+	+	+	-----	-----	-----	-----	-----
Hagonoy	—	±	—	—	-----	-----	-----	-----	-----
Orani	—	—	-----	—	-----	-----	-----	-----	-----
Navotas A	—	—	-----	—	-----	-----	-----	-----	-----
Navotas B	-----	-----	-----	-----	-----	-----	-----	-----	-----
136 B	—	±	-----		-----	-----	-----	-----	-----
136 A	—	±	-----	—	-----	-----	-----	-----	-----
King A	—	-----	-----	-----	-----	-----	—	—	-----
Gapus	—	—	-----	—	-----	-----	-----	-----	-----
B. Robles	—	-----	-----	—	-----	-----	-----	-----	-----
Carolina	—	—	—	—	—	-----	-----	—	
Bilibid	—	—	—	—	—	-----	—	—	—
869	—	—	—	—	-----	-----	-----	-----	-----
568	—	—	—	—	-----	-----	-----	-----	-----
371	—	—	—	—	-----	-----	-----	-----	-----
75	—	+	+	+	±	—	-----	—	—
48	—	±	—	—	-----	-----	-----	-----	-----
21	—	—	-----	—	-----	-----	-----	-----	-----

Agglutination.—Various observers have noted a delayed or slow agglutination and spontaneous agglutination of certain strains. The results of agglutination usually were noted by us after the tubes had been placed for one to two hours in the thermostat at 37°, and for three hours at room temperature. One cholera strain reached the limit of agglutination only after twenty-four hours; two others only after twelve hours; the rest were positive within three hours. With all fresh cholera strains the results could be noted within three hours. One noncholera and one cholera strain were discarded because of their tendency to spontaneous agglutination. These organisms had been kept upon laboratory media for many months.

We found no instance of spontaneous agglutination, delayed agglutination, or diminished agglutination when working with fresh cholera strains, but in every instance they agglutinated promptly to the limit of the specific serum and showed no tendency to spontaneous agglutination in salt solution or serum other than specific cholera serum.

Marked differences in the agglutinability of various cholera strains of similar virulence usually does not exist. However, the four strains, Abalogan, Berezosa, León and Solitana, required for agglutination much stronger concentrations of cholera sera than the other cholera strains.

These were supposed to be strains of true cholera. They had been isolated from typical cholera cases and agglutinated in the routine manner by cholera serum in a dilution of 1–200. They were kept in ordinary agar medium and transplanted about once each month during July, August, September, and October. In November, cholera serum Regala II agglutinated all of the other cholera strains in a dilution of 1–2,000 + and did not agglutinate the four above mentioned in any dilution weaker than 1–200.

These cultures were carefully plated and the serum test repeated, with the same result. Each of the four produces in a rabbit a serum which agglutinates the homologous vibrio; the other three strangely acting vibrios, and all the cholera strains, in dilutions of from 1–1,000 to 1–2,000.

Four new cholera sera were prepared from the freshly isolated cholera strains, numbered 2, 4, 5 and 6, and the results may be seen in Table II. With these fresh sera (numbered 2, 4, 5, and 6) it will be noted that the strains Abalogan, León, and Solitana were not affected in the same manner as other cholera strains, although the differences were not so great as that shown by serum Regala II.

The lowered agglutinability of these four strains was not in evidence with all cholera sera. With the four homologous sera, and with one of the *El Tor* sera (King B), these strains were agglutinated to the limit.

It can not be said that the agglutinability always bears a fixed relation to the virulence of an organism. Old, avirulent laboratory cultures, Pfeiffer, Hankin, etc., are particularly susceptible to cholera sera; agglutinating in weaker dilutions than recently isolated strains.

HAEMOLYTIC PROPERTIES.

The hæmolysing quality of certain vibrios has been the cause of much discussion.

Ruffer (10) upon this group considers the *El Tor* vibrio to be distinct from cholera, as none of his cholera strains possessed hæmolysing properties. Kolle and

Meïnicke(5), Muhlens and Von Raven(7), Schumacker(11), Neufeld and Hændel(8), demonstrated that certain cholera strains possessed hæmolysing properties. These workers used cultures which had been kept for a considerable time upon laboratory media. Kraus(6), working with fresh cholera cultures, was unable to find a strain with hæmolysing properties.

The hæmolytic action of our vibrios was tested by the simple method employed by Ruffer and also by the method of Kraus. One loop of an eighteen-hour culture was suspended in 5 cubic centimeters of normal salt solution. To 0.5 cubic centimeter of this suspension, 0.5 cubic centimeter of a 5 per cent suspension of well-washed blood corpuscles was added. The mixture was placed in the thermostat at 37° and results noted after two hours, four hours, and twenty-four hours. This method was also used by Neufeld and Hændel. By this means Ruffer found no strain of cholera with hæmolytic properties, while Neufeld and Hædel found that several cholera strains were capable of producing hæmolysis. However, none of these were freshly isolated.

Our cholera strains, both fresh and old, uniformly failed to produce hæmolysis with these methods. Both *El Tor* strains examined were hæmolytic, as well as 18 of the 22 noncholera vibrios. The results are shown by Table III.

TABLE III.—*Hæmolytic tests.*

NONCHOLERA VIBRIOS.

Name of culture.	Ruffer's method, suspension of live vibrios.			Kraus's method, 0. 1 cc. 3-day bouillon, killed to 1 cc. 5 per cent suspension red cells.		
	2 hours.	4 hours.	24 hours.	2 hours.	4 hours.	24 hours.
Iris	+	+ +	+ + +	--------	+	+ +
Notbil	+	+ +	+ + +	--------	+	+ +
Sande	--------	+	+ + +	--------	+ +	+ + +
Malabon	+	+ + +	--------	--------	+	+ +
Sta. Monica	+	+ +	+ + +	--------	+ +	+ + +
Hagonoy	+	+ +	+ + +	--------	+ +	+ + +
Orani	—	—	—	--------	—	—
Carolina	—	—	—	--------	—	—
135	—	—	—	--------	—	—
Navotas A	+	+ +	+ + +	--------	tr.	+
Navotas B	+	+ +	+ + +	--------	tr.	+
Sandejas A	+	+ +	+ + +	--------	tr.	+
Sandejas B	+	+ +	+ + +	--------	tr.	+
Gapus	+ +	+ + +	--------	tr.	+ +	+ + +
Bilibid	—	+	+ +	—	tr.	+
869	--------	+	+ + +	--------	+	+ +
846	+	+ +	+ + +	--------	+	+ +
568	--------	+	+ + +	--------	+	+ +
371	—	—	—	—	—	—
75	+	+ +	+ + +	--------	+	+ +
48	+	+ +	+ + +	--------	+	+ +
21	--------	+	+ + +	--------	—	+

TABLE III.—*Hæmolytic tests*—Continued.

CHOLERA VIBRIOS.

Name of culture.	Ruffer's method, suspension of live vibrios.			Kraus's method, 0.1 cc. 3-day bouillon, killed to 1 cc. 5 per cent suspension red cells.		
	12 hours.	24 hours.	48 hours.	12 hours.	24 hours.	48 hours.
King A (El Tor)	+ +	+ + −				
King B (El Tor)	+ +	+ + −				
Abalogan	−	−	−			
Berezosa	−	−	−			
Leon	−	−	−			
Solitana	−	−	−			
1131	−	−	−			
1108	−	−	−			
1101	−	−	−			
1101	−	−	−			
1051	−	−	−			
1038	−	−	−			
1029	−	−	−			
955	−	−	−			
954	−	−	−			
953	−	−	−			
952	−	−	−			
1021	−	−	−			
758	−	−	−			
664	−	−	−			
622	−	−	−			
545	−	−	−			
492	−	−	−			
Cavite I	−	−	−			
Cavite II	−	−	−			
Lorenzo C	−	−	−			
Santiago	−	−	−			
No. 4 Taytay	−	−	−			
Miraflores	−	−	−			
Bautista	−	−	−			
B. Robles	−	−	−			
Suing	−	−	−			
No. 30	−	−	−			
Salus	−	−	−			
Regala I	−	−	−			
Regala II	−	−	−			
David	−	−	−			
Nicolas	−	−	−			
Camay	−	−	−			
Ramon	−	−	−			
Sucero	−	−	−			
C. Robles	−	−	−			
Suegang	−	−	−			
Dizon	−	−	−			
Aniecte	−	−	−			
Maquio	−	−	−			
Calderon	−	−	−			
No. 2	−	−	−			

TABLE III.—*Hæmolytic tests*—Continued.

Name of culture.	Ruffer's method, suspension of live vibrios.			Kraus's method 0.1 cc. 3-day bouillon, killed to 1 cc. 5 per cent suspension red cells.		
	12 hours.	24 hours.	48 hours.	12 hours.	24 hours.	48 hours.
No. 3	—	—	—			
No. 4	—	—	—			
No. 5	—	—	—			
No. 6	—	—	—			
902	—	—	—			
Betonio	—	—	—			
Caballeros	—	—	—			
Mendoza	—	—				
046	—	—	—			
074	—	—	—			
099	—	—	—			
125	—	—	—			
Zacarias	—	—	—			
Salvador	—	—	—			
Tarlac	—		—			
Lloret	—	—	—			

DOES THE HOG ACT AS A "CHOLERA CARRIER" IN THE PHILIPPINES?

Because of the extent to which the hog acts as a scavenger in this Archipelago, it seemed advisable to determine whether or not that animal could act as a cholera carrier under natural conditions. Accordingly, two partly grown pigs were selected for feeding, while a third was chosen as a control.

After carefully ascertaining that there were no cholera-like vibrios in the stools of any of the animals, two were fed three twenty-four hour agar slants of a recently isolated cholera vibrio, mixed with fæces from a normal man and made faintly alkaline with sodium carbonate. The stools of these two pigs were examined daily for a week, but we were never able to isolate a vibrio from them. The experiment was repeated on the same animals with the same result. Finally, each one of the pigs was fed a litre of fresh rice water stool from cases of cholera in San Lazaro Hospital. Neither showed any bad effects from the feeding and again we were unable to isolate a vibrio from the fæces from either pig in an examination made each day for ten days. As we were endeavoring to simulate natural conditions, we did not try the effect of neutralizing the contents of the pigs' stomachs, nor similar procedures.

SUMMARY.

We have not been able to add anything to what is already known to questions numbered (1) and (2). Every vibrio in our list which agglutinates in a 1 to 200 dilution of specific anticholera serum is a cholera vibrio, while none of the noncholera vibrios agglutinated in a weaker dilution than 1 to 10. On the other hand, four of our cholera vibrios did not agglutinate equally with all of our cholera sera; with some they did not agglutinate in a weaker dilution than 1 to 200. It is noted that three of these vibrios had been in mixed cultures on agar for about seven days, in transit from the provinces, but whether this had anything to do with their lowered agglutinability we are not prepared to state.

None of our agglutinating strains were hæmolytic, so that we have not found any vibrios of the *El Tor* type in these Islands.

We have not been able to make "cholera-like" vibrios acquire agglutinability with cholera sera.

Dieudonné's medium is satisfactory for the isolation of cholera vibrios from the stools, but it does not assist in separating cholera vibrios from "cholera-like" vibrios.

We have not been able to show that the hog acts as a "cholera carrier" under as nearly natural conditions as possible.

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THE SPECIFIC CURE OF YAWS WITH DIOXY-DIAMIDO-ARSENOBENZOL.[1]

By Richard P. Strong.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

In certain portions of the Philippine Islands yaws, or frambœsia,[2] is a very common disease. In some of the smaller towns lying near Manila, for example Parañaque, Las Piñas and Bacoor, it occurs much more frequently in children than in adults, a considerable percentage of the former being infected. In the course of a search of an hour or two I was able to find in the neighborhood of a dozen or more cases of this disease in each of these villages.

The disease is not only highly disfiguring, but it is very contagious and usually runs a chronic course, frequently preventing the patient from working and sometimes eventually disabling him. The treatment hitherto has been most unsatisfactory and usually must be very prolonged before success is obtained. While cures after long periods have frequently resulted among the better classes affected, as a rule the disease is one of very long duration and yields but slowly to medication. Some of the cases reported in this paper demonstrate these points. Even the patients who can be seen in the dispensary and hospital as a rule become discouraged with the treatment long before a cure results, and usually abandon it. The successful treatment of this disease in the cases among the lower classes frequently encountered in the smaller towns hitherto has been entirely impracticable, for the reason that it usually has been impossible to persuade and also to cause individuals to take a prolonged course of medication, which, to be successful ultimately, frequently must be pursued for many months.

Manson states that yaws runs a chronic course and lasts for weeks, months, and years. The duration depends on the general health and idiosyncrasies of the patient as well as on his hygienic conditions and the treatment employed.

According to Daniels the usual duration of the disease is two or three years, but on the parts of the body where the epidermis is thick, such as the soles of the feet, it may persist for much longer periods.

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[1] Preliminary remarks on this subject were made before the Manila Medical Society on September 5, 1910, where photographs and one of the cured patients were presented.

[2] Commonly known among the natives as *bubas*.

Scheube believes that the duration of yaws varies between a few weeks and several years, and that the treatment, food and hygienic condition of the patient influences the time.

According to Castellani, unless the disease becomes extinct after the secondary stage, it may extend to many years. Jeanselme states that framboesia runs a chronic course varying from several weeks or months to a number of years. Firth describes yaws as a chronic disease, the secondary eruption lasting from a few months to two years.

THE SPECIFIC CHARACTER OF YAWS.

It is not my intention here to enter into a prolonged discussion of the question of the specificity of yaws as a disease. Almost all observers agree that it is not identical with syphilis. Sheube and Firth are inclined to support the original idea of Hutchinson that yaws was the original disease from which European syphilis has been evolved or that the two diseases developed from a parent form, each having been modified by being propagated by different races in different climates. Manson, Castellani, Jeanselme and Plehn regard them as specifically distinct.

I have had abundant opportunity for the observation of all forms of yaws in several tropical countries for a number of years and my own opinion, based largely upon clinical material, histologic examinations, experiments in inoculation and serum reactions carried on in this laboratory, as well as upon the experimental work of others, is strongly in favor of the view that yaws and syphilis are distinct.

I believe that while the two diseases are certainly closely related from an etiologic standpoint, yet they are different affections. Since the discovery of the specific organism of yaws, *Treponema pertenuis,* by Castellani in 1905, a number of observers have carried on comparative morphologic studies between this organism and *Treponema pallidum.*

Prowazek, Blanchard, Martin and Levaditi have discussed the slight morphologic differences between these organisms; these however are not sufficiently constant or definite to serve as a means of differentiation. On the other hand, inoculation experiments, both in man and animals, appear to have resulted in a more definite distinction. Charlouis in 1881 inoculated a native suffering from typical yaws, with syphilis. A primary indurated syphilitic chancre developed, and a typical secondary syphilitic eruption followed. This experiment apparently has not been repeated in man, although a number of observers, Bestion, Powell, A. Nicholls and others, have reported cases of yaws which subsequently have contracted syphilis.

Neisser, Baermann, Halberstädter, and Castellani have shown that monkeys successfully inoculated with framboesia do not thereby become immune to syphilis, and subsequently may be infected with the latter disease and vice versa, apes successfully inoculated with syphilis remain susceptible to framboesia. Halberstädter also believes the appearances of the primary inoculative lesion in lues and framboesia to be different. All of these authors conclude from their experiments that the two diseases are etiologically distinct.

Levaditi states that monkeys immunized against yaws do not acquire any immunity for syphilis, but monkeys immunized against syphilis acquire partial immunization for frambœsia. Ashburn and Craig found that monkeys of the species *Cynomolgus philippinensis* Geoff. are susceptible to yaws, but not to syphilis.

Castellani applied the Bordet-Gengou complement fixation reaction to yaws and concluded from his experiments that it was possible to demonstrate the existence of specific yaws antibodies and antigen. The technique he employed was similar to the usual one recommended by Wassermann, Neisser and Bruck. Goat's corpuscles and rabbit's hæmolytic serum were used. When extracts of yaws papules were added to serum from monkeys which had been inoculated successfully with yaws, the reaction was positive. When the reaction was performed with extract of syphilitic condylomata, or extract of a primary syphilitic sore (in place of an extract of yaws papules) and with yaws serum, the reaction was negative; also, when performed with extract of yaws papules and with serum from a monkey immunized for syphilis, the reaction was negative. From these and other experiments, Castellani concluded that it is possible to detect specific antigen in the yaws papules and in the spleens of cases of yaws; and specific yaws antibodies in the blood of monkeys treated by inoculation with yaws material; also that yaws antibodies and antigen are different from syphilitic antibodies and antigen, and therefore syphilis and yaws differ specifically.

Bowman, in this laboratory, also performed a few experiments with the complement binding reaction in cases of ayaws and syphilis. He found in the usual way as suggested by Landsteiner, Neisser and Poetzl, that when an extract of guinea pig's heart was added to the serum from a case of syphilis, the reaction was positive. Red blood cells of the ox and immune hæmolytic rabbit serum were employed. On the contrary, when the extract of the guinea pig's heart was added to a serum from a human case of yaws, the reaction was negative. When extract of yaws papules was added to serum from yaws patients, the reaction was positive. Bowman concludes that the serum from cases of yaws does not bind complement when the extract from a guinea pig's heart is used, and that his experiments furnish additional evidence of the nonidentity of syphilis and yaws.

Hoffman, on the other hand, reported a case of yaws in which Blumenthal found the Wassermann reaction positive. Ziemann did not think the diagnosis of this case definite.

There seems little doubt but that cases of syphilis have sometimes been diagnosed as those of yaws, and in some of the recent literature pictures are found of cases diagnosed as yaws in which the lesions appear to resemble rather those of syphilis and in which the diagnosis of yaws from the pictures would seem hardly justified.

While this article was in the course of preparation, H. Nichols' articles in the "Journal of Experimental Medicine" and "Journal of the American Medical Association" were received. Nichols reports that rabbits can be infected in the testicle with the spirochætæ of yaws, and that the complement fixation reaction has been tried on rabbits so infected and has been positive in several cases.[3] In the experiment he employed acetone insoluble liver extract as antigen, and the human corpuscle and rabbit hæmolytic system. On the other hand, the reaction failed in his hands in the case of a negro suffering from yaws and in two monkeys infected with the same disease.

Bruck reported a positive Wassermann reaction in a patient who gave a history of having had yaws five years before. However, it appears that the observer would hardly be justified in drawing definite conclusions in regard to the diagnosis of this case since the lesions might have been those of syphilis.

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[3] He also reports the favorable influence of dioxy-diamido-arsenobenzol on the experimental lesions produced in the testicles of four of these rabbits.

Until we have more information on the question of the complement binding reaction, it would be unwise to draw any absolute conclusion from the result of the experiments with it in relation to yaws and syphilis.

However, from the other data submitted it is believed that the specificity of yaws as a disease has been shown and it is not deemed necessary here to present further the evidence based upon clinical and pathologic observation.

TREATMENT FORMERLY RECOMMENDED.

The consensus of opinion of those who have had a wide personal experience with yaws is that the treatment of the disease is usually unsatisfactory.

Manson writes:

> All are agreed as to the propriety of endeavoring by good food, tonics and occasional aperients to improve the general health. Most are agreed as to the propriety of endeavoring to procure a copious eruption by stimulating the functions of the skin by warm demulcent drinks; by a daily warm bath with plenty of soap; and, during the outcoming of the eruption, by such diaphoretics as liquor ammoniæ acetatis, guaiacum, etc. Confection of sulphur is also recommended as a suitable aperient; it may be taken frequently in the early stages of the disease. All are agreed as to the propriety of avoiding everything—such as chill—tending to repress the eruption; warm clothing is therefore indicated. Many use mercury, or potassium iodide, or both, after the eruption is fully developed. These drugs have undoubtedly the power of causing the eruption in yaws to resolve. Some practitioners rarely use them, or, if they do so, only at the latest stages of the disease considering that relapses are more prone to occur after their too early employment. Mercury, owing to its proneness to cause anæmia, is less frequently employed than potassium iodide. Where the eruption is persistently squamous, or papular, arsenic is frequently prescribed. Some touch the yaws with sulphate of copper; some apply nitrate of mercury ointment; others iodoform ointment; others leave them alone, confining their local measures to the enforcement of cleanliness. When the soles of the feet are attacked, the feet ought to be soaked in warm water to soften the epidermis, which should then be cut away sufficiently to liberate the subjacent yaw. Ulceration must be treated on ordinary principles. During convalescence, iron, arsenic and quinine are indicated.

Scheube favors internal treatment with mercury and iodide, and states that Schuffner has communicated to him that the small number of cases which do not yield to such treatment are usually very favorably influenced by Zittmann's decoction. After antisyphilitic treatment, arsenic is recommended, particularly in weak and anæmic patients. Local solutions of sublimate, and touching with carbolic acid, nitric acid, mercuric nitrate, copper sulphate, silver nitrate, applications of tincture of iodine, iodoform ointment or oil, and mercuric salve are recommended. In cases with excrescences of long duration, curetting with a sharp spoon, or removal with the scissors, is also advised. Scheube mentions that Mense found a watery paste of bismuth subnitrate most successful for local application, while Hirsch recommends one part of lactic acid and eight parts of nitrate of mercury ointment for this purpose.

Plehn states that mercury and iodide should in all cases be tried. Cauterization of the lesions with a hot iron or sharp curetting of them is not recommended. Bismuth paste is preferred for local treatment. In native children calomel powder is advised for internal administration several times daily in doses of 0.002 to 0.01 gram, according to the age. In case the eruption is not too extensive, it is recommended to cover the lesions with calomel powder or grey salve. Painting with a thin paste of calomel and salt water is also advised. For ulcerating granulomata about the lips or corners of the mouth an alcoholic sublimate solution 1 to 100 or 20 to 30 per cent chromic acid solution is recommended. A continuation of the treatment after disappearance of the lesions, in order to prevent their return is not thought to be necessary.

Castellani states, the long duration of frambœsia and its great contagiousness render it a serious malady. Patients suffering from it are unable to attend to their work. In Ceylon the majority of European practitioners use mercury and potassium iodide. Others affirm these drugs to be quite useless, and believe that cleanliness and good and abundant food are quite sufficient to bring about a cure. In Castellani's clinic the conclusion arrived at was that potassium iodide was the most effective means of treatment. He states that some cases may recover spontaneously, but this is certainly the exception, not the rule. The potassium iodide should be given in large doses; in adults, 15 grains (1 gram) and in children 5 grains (0.33 gram), three times daily. Occasional cases are met with refractory to treatment, and tertiary lesions are often intractable. Mercury is of very little use as a rule. In some very stubborn cases a course of potassium iodide, followed by one of atoxyl injections is recommended. Castellani further states: 'An important point often overlooked by the practitioner is that the treatment should be prolonged for a considerable time after the complete disappearance of the eruption, inasmuch as clinical experience, as well as experiments on inoculated monkeys, prove that the specific treponemata may, and do persist in the lymphatic glands and internal organs long after the cutaneous manifestations have disappeared. Local treatment consists chiefly in keeping the skin scrupulously clean, washing the eruption twice daily with a perchloride of mercury solution (1 to 1,000) which greatly allays the itching. The ulcerated lesions may be dusted with iodoform, euruphen, or boracic acid. Mercury ointments may be beneficial, but in our experience are not sufficient to hinder secondary pyogenic infections. Caustics are not called for unless the ulcers become phagedenic. In such cases pure carbolic acid is best. Though the external treatment may be useful, one must bear in mind that it is not, as a rule, sufficient alone to cure the disease.'

Jeanselme believes that mercurial treatment has a certain influence upon yaws, but it does not prevent relapses. The author treated sixteen children from 3 to 12 years of age with mercury. They took regularly during fifteen days from two to four teaspoonfuls of "liqueur de Van-Swieten," according to the age of the child. The lesions dried up rapidly, and became covered with thin, sulphur-yellow crusts. When the author was obliged to terminate the experiment, five of the patients had a clear skin; the lesions in nine patients were visibly ameliorated; only two of the children did not receive any benefit from the treatment. Dressings and lotions of sublimate are said to influence very favorably the eruption. Jeanselme states that potassium iodide employed in the same doses as in syphilis has an unquestionable action upon yaws. It causes the eruption rapidly to recede. In obstinate cases cauterization of the vegetative ulcerations with sulphate of copper, with acid nitrate of mercury, and scraping with a curette, following with igneous cauterization, gives good results.

Firth states the first essential in the actual treatment of the attack to be the cleansing of the patient by means of warm baths and soap. Special care must be taken to avoid chills, as exposure to cold often causes a disappearance of the eruption, accompanied by much constitutional disturbance. The food must be nourishing, consisting of fresh meat, fish, rice, yams, and diluent drinks, combined with medicinal tonics. Locally, disinfectant lotions of boric or carbolic acids, or of corrosive sublimate, are of the first importance. The acid reaction of the secretion from yaws tubercles has suggested the use of alkali as a local dressing. Both Modder and Rat state that excellent results follow the use of lotions containing either bicarbonate of soda or carbonate of ammonia of the strength of ten grains to the ounce. Sulphate of copper is efficacious as a topical application; so likewise are iodoform and weak nitrate of mercury ointment. The use of mercury in this disease needs the utmost care and supervision, as its abuse has been largely responsible in the past for the severity and fatality of many cases; *it can not be regarded as a specific remedy for the disease, as it is for syphilis*, but, given in minute doses for a short time, mercury seems to act as a beneficial alterative. Of other internal remedies, iodide of potassium with arsenic is very valuable, while in some cases iron and sulphocarbolate of calcium are of the greatest benefit. Arsenic is very successful in the cases in which the eruption is badly developed or scaly, as in the *pian darte* variety. When the feet and hands are affected, prolonged soaking in hot water is often required in order to soften and remove the thick epidermis; the exposed yaws growth can then successfully be treated on the lines indicated above. During convalescence iron and arsenic should both be administered over long periods; while in all stages of the affection perfect cleanliness and the best hygienic conditions are needed, both for the sake of the sick and of those brought into contact with them.

Woolley recommends potassium iodide internally and local applications of bichloride of mercury followed by iodoform.

Daniels states that probably no drug influences the duration of the disease. Mercury and arsenic certainly do not. Potassium iodide is uncertain in its action. The eruption will sometimes disappear rapidly when iodides are given, but even in such cases when the use of the drug is continued, fresh eruptions appear. The use of iodide is therefore limited. Local applications that merely serve to keep the granuloma clean are valuable, but escharotics and irritants, although they may destroy the yaws, are likely to cause the formation of scars. The painful granulomata on the feet are best removed by the action of nitric acid, acid nitrate of mercury, or silver nitrate.

From this summary of the literature of the treatment of the disease, it appears that a number of eminent authors regard its local treatment as very important, and that even the most successful drug (potassium iodide) frequently fails to produce a cure, and that in many cases the treatment of yaws, to be successful, must be a very prolonged one.

SPECIFIC TREATMENT OF YAWS.

A few months ago, through the kindness of Professor Ehrlich, I received a shipment of dioxy-diamido-arsenobenzol[4] for use for experimental purposes in syphilis. I take great pleasure in publicly acknowledging my thanks to him for the opportunity to try the effect of this very valuable preparation. Because of the favorable results which were being obtained with it in the treatment of syphilis, the idea naturally suggested itself to try the remedy in yaws on account of the morphological and biological relationship which exists between the spirochætæ giving rise to these two diseases. Twenty-five cases in all have been studied and treated. A report of the cure of three of the most striking of these was made before the Manila Medical Society on September 5, where photographs and one of the patients were exhibited. The discussion of all of the work has been withheld until the present time, in order to ascertain if relapses would occur. So far there has been no evidence of a relapse, and the cases have all remained cured, although the great majority of them were treated over four months ago.

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[4] $(NH_2 . OH . C_6H_3 . As : As . C_6H_3 . OH . NH_2)$.

The preparation was received in hermetically sealed capsules. These were opened, and the solution always prepared immediately before being inoculated. Dioxy-diamido-arsenobenzol, as Ehrlich has stated, is not readily soluble, and to obtain a satisfactory solution for inoculation, his suggestion as to the most satisfactory means for the solution of the monochlorhydrate was followed. From 0.3 to 0.4 gram of the preparation was placed in a glass vessel, moistened with a few drops of methyl alcohol, and thoroughly rubbed up with a glass rod. From 20 to 30 cubic centimeters of distilled water were then added, the stirring with the glass rod being continued. When the solution was apparently complete, 5.5 cubic centimeters (for 0.3 gram of the preparation) of an $\frac{N}{10}$ sodium hydroxide solution were slowly added, stirring with the glass rod being continued. The precipitate, which forms on this addition, was then slowly redissolved.

The injections were given deep into the gluteal muscles, from 15 to 20 cubic centimeters of fluid usually being injected in one place. On the following day and the day after there was usually some reddening, swelling and induration of the skin and underlying tissues in the vicinity of the point of the inoculation. In no instance did suppuration result, nor any unfavorable symptoms develop. Occasionally a transitory rise of temperature was noted a few days after the injection. In some of the cases, pain was complained of after the injection, sometimes lasting for from 10 to 24 hours, or sometimes even longer, but as a rule no great complaint was made. A number of the inoculated cases have been among young children who did not cry, either during the inoculation or after it; usually they did not care to play about for the first 24 hours following the injection. One European suffering with syphilis who was given a large injection into the buttock, suffered severe pain, lasting for nearly 24 hours. This case was given a second injection intravenously twenty days later. No pain whatever followed.

It was found that in every instance for the cases of yaws treated a single injection of the drug was sufficient to produce a cure, even though a number of them showed tertiary lesions with ulcerations. No local treatment was used with any of the patients, nor was any other treatment internal or otherwise administered after the injection. A few cases had previously been treated with iodides; these were discontinued a few days before the injection of dioxy-diamido-arsenobenzol was given.

NOTES OF THE CASES TREATED.

The notes of the cases treated are as follows. Photographs before and after treatment frequently were secured where opportunity was favorable.

Case I.—(Plates I and II.) Felixberto; male, age 11 years. Diagnosis, yaws secondary stage; duration of the disease, about one year.

The lesions are situated anteriorly over the forehead, nose, cheeks, upper lip, chin, chest, upper arms and forearms, thighs and legs. These consist of raised, mushroom-like, sharply circumscribed granulomata measuring from 1 to 5 centimeters in diameter and about 1 to 1.5 centimeters in thickness. They are in general circular in outline. Frequently the lesions have coalesced. On the right ankle is an excoriated lesion apparently the result of traumatism, and on the right forearm just above the wrist is an ulcerated granuloma. The patient has been under treatment at the St. Luke's dispensary for the past two months. For about seven weeks he has taken five grains of potassium iodide three times daily. Before this he took syrup of iodide of iron. Locally, he was treated with bichloride and salicylic acid dressings, these being applied every few days. The treatment has apparently had no effect whatever on the disease. A smear from one of the granuloma stained with Giemsa's solution showed a few spirochætæ. Plate I shows the patient as he appeared after two months of the treatment as described above. All of these measures were then discontinued and the patient given an injection of a solution of 0.28 gram of dioxy-diamido-arsenobenzol, half of the solution being injected into each buttock. On the fourth day after the injection it was reported that the lesions were disappearing. The patient was seen by the author one week after the injection. The granulomatous lesions had almost entirely disappeared. In twelve days the skin was perfectly smooth; the lesions had entirely disappeared, and only pigmentation of the skin remained where the granulomatous lesions formerly existed. Plate II represents a photograph taken at this time. The skin was everywhere perfectly smooth. Examination of the urine one week after the injection showed it to be normal. The case since has remained entirely well.[5]

Case II.—(Plates III and IV.) Gonzales; male, age 5 years; duration of the disease 8 months. The lesions are scattered over almost the entire face, neck, upper arms and forearms, hypogastric region, thighs, legs, ankles, and feet; posteriorly over the neck, shoulders, arms and forearms, back, buttocks, thighs, legs and ankles. The chest and abdomen are particularly clear. The lesions consist of granulomata measuring usually from about 0.3 to 1.5 centimeters in diameter, being slightly raised above the skin and being covered, particularly on the face by yellow crusts. On the arms, forearms and legs, the lesions are more distinctly papular in character. A smear from one of these papules shows spirochætæ. This child had been treated previously with native remedies but with no favorable results (see Plate III). He was given an injection of 0.25 gram dioxy-diamido-arsenobenzol dissolved in 20 cubic centimeters distilled water, one-half of the amount being injected deeply into each buttock. Nine days later the lesions had completely disappeared, as may be seen from Plate IV, which was taken at that time. The skin was then everywhere clean and smooth, and only pigmented patches, more marked on the legs and arms, remained as an evidence of the pre-existing lesions. There has been no relapse, and the patient since has remained entirely well.

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[5] I am indebted to Dr. Eleanor Pond of St. Luke's Dispensary for having notified me of this case.

Case III.—(Plates V and VI.) Fausto; male, age 9 years; states duration of the disease about two months. The lesions are situated on the forehead, neck, shoulders, left arm, at the bend of each elbow, forearms, thighs and legs, and posteriorly over the back, buttocks, thighs, popliteal spaces, legs and ankles. They consist of granulomatous tumors and papules sharply circumscribed, measuring from about 4 or 5 millimeters to about 4 centimeters in diameter. On the forehead and neck the lesions measure about 4 centimeters in diameter, and are distinctly granulomatous and raised for a distance of about 0.5 centimeter above the surrounding skin. The same character of lesion, although smaller in diameter, occurs at the bend of the elbows and over the region of the left popliteal spaces; elsewhere the lesions are more distinctly papular in character. On the left heel is an ulcerating granulomatous lesion (see Plate V). A smear from one of the papules shows spirochætæ. There are two other members of this boy's family afflicted with yaws, the father and brother. The brother's condition is reported as Case No. IV. The present patient was injected with 0.3 gram of dioxy-diamido-arsenobenzol in 20 cubic centimeters distilled water, the injection being given into the buttocks in the usual manner. Nine days later the lesions had practically disappeared. Dry, scaly patches remained in the left popliteal space, and anteriorly at the bend of the elbows and over one spot on the neck. The lesion on the left foot was covered by a clean scab. These scaly patches had disappeared, and the ulcer on the heel entirely healed, five days later. (See Plate VI.) The case since has remained entirely well.

Case IV.—(Plates VII and VIII.) Enrique; age 4 years; brother of Case III; duration of disease 2 years. The lesions are situated particularly about the lower lip and chin, the right axilla, bends of the elbows, forearms and thighs, and posteriorly in the right axillary fold, at the bends of the elbows, the gluteal fold, buttocks, popliteal spaces and legs. They consist of ulcerating granulomatous masses measuring 5 or 6 centimeters in diameter, raised in some instances a centimeter and a half above the surrounding skin, and partially covered by yellow crusts. There are large scars over the back of the legs, right thigh, ankle, and numerous small pigmented patches on the legs, arms and a few on the chest, the results of healed lesions (see Plate VII). Smears from the ulcerating lesions reveal several forms of bacteria and spirochætæ. The patient was given an injection of 0.28 gram of dioxy-diamido-arsenobenzol. Nine days later a very remarkable change had taken place in the lesions. These on the arms and legs were very greatly reduced in size, but had not entirely disappeared. However, the appearance of the remaining lesions was entirely unlike that of granulomata, they being composed mostly of contracted scar tissue. The lesions about the mouth had almost disappeared, a thin scab being visible on the chin. Some of these changes may be noted in the photograph, Plate VIII, fig. 1. Unfortunately, the photographer had not another plate with him so that no posterior view of the patient could be taken at this time. The patient was seen twelve days later and again examined and photographed (see Plate VIII, figs. 2 and 3). The lesions were then almost healed with the exception of a small scab at the bend of the left elbow anteriorly and another posteriorly, and a scab over the right popliteal space. The skin since has become entirely clean and the patient has remained well. The lesions in this case were of a most severe character.

Case V.—Apolonia; female, age 9 years; mother also infected (see Case IX); duration of the disease 4 years. The lesions consist of an ulcerated granuloma over the left ankle, apparently a tertiary lesion. Over the right internal malleolus there is a large fungating mass with much scar tissue about it; there are also granulomata about the toes and many scars and pigmented patches on the right leg. Above the knee on the left thigh there is a large scar measuring about 10 centimeters in diameter. There is also a large scar on the outside of the knee of the right leg. Over the chest, abdomen, and back there are numerous scars and patches of pigmented skin where previous lesions have apparently existed. Spirochætæ were present in a smear from one of the lesions. The patient was given 0.3 gram of dioxy-diamido-arsenobenzol, injected as usual into the buttocks. She could not be found nine days later, but was seen twenty days after the injection, when the granulomatous lesions had entirely healed, and she since has remained well.

Case VI.—Teodorica; female, age 6 years; duration of the disease 3 years. The lesions consist of an ulcerating granuloma on the left heel; pigmented patches over the buttocks, posterior surface of the left leg, and the abdomen. Over the sternum there is a scar measuring about 4 centimeters in diameter. There are also large scars over the right side of the neck, which are reddened and have evidently recently healed. There are two granulomata measuring about 3 and 4 centimeters in diameter on the right side of the neck and one of the left forearm measuring about 3 centimeters. A smear from one of these shows spirochætæ. The patient was given 0.28 gram of dioxy-diamido-arsenobenzol in the usual manner. Fifteen days later the granulomatous lesions had entirely disappeared. On the heel was a small clean scab. When seen one week later the heel also was perfectly clean. There has been no return of the eruption.

Case VII.—Ciriaco; male; age 16 years; duration of disease ten months. The lesions consist of an ulcerating granuloma situated above the right ankle, and measuring about 6 centimeters in diameter. Over the left external malleolus there is a small ulcerating patch measuring about 3 centimeters in diameter. There is also a small ulcerating granuloma over the left great toe. A smear from this shows spirochætæ and a few bacteria. There are pigmented patches scattered over the skin of the whole left leg and over the thigh. These patches are evidently the results of healed lesions. The chest and back are clear. The patient was given 0.3 gram of dioxy-diamido-arsenobenzol in the usual manner. He was seen nine days later, when the lesions had almost disappeared. Twenty days later they had entirely healed. He has since remained well.

Case VIII.—Buenaventura; age 10 years; duration of disease stated to be eight months. The lesions consist of a granulomatous ulcer situated above the left ankle, measuring about 5 centimeters in diameter. There are smaller ulcerated granulomata over the right ankle. There are pigmented patches over the knees and over the back of the thighs and legs. On the neck there are four papular tumors measuring about 1 centimeter in diameter. A smear from one of these shows spirochætæ. The patient was given 0.3 gram of the preparation in the usual manner, and when seen twenty days later the skin was smooth and the active lesions were entirely healed; there has been no return of them.

Case IX.—Maria; age 25 years; the mother of Case V; duration of disease (?). The lesions consist of four granulomata ranging from 2 to 4 centimeters in diameter, and situated on the buttocks and one within the gluteal fold. A smear from one of these revealed spirochætæ. The patient was injected with 0.4 gram of the preparation in the usual manner. Twenty days later the active lesions had entirely disappeared, leaving pigmented patches of smooth skin where they had formerly existed.

Case X.—Bernardo; male; age 22 years; duration of disease one and one-half years. There is an ulcer measuring 3 centimeters in diameter, evidently a tertiary lesion, situated on the left ankle and a small fungating mass on the left toe. There are numerous pigmented patches and scars over the posterior surface of the legs and back. Spirochætæ were found in a smear from the fungating mass. The patient was given 0.4 gram of the preparation by injection in the usual manner. Twenty days later the lesions had entirely healed. He since has remained well.

Case XI.—Tomas; age 11 years. The lesions consist of about thirty papillomata situated over the shoulders, arms, forearms and knees. They are sharply circumscribed, and measure from about 4 millimeters to 1 centimeter in diameter. A smear from one of these shows a few spirochætæ. Three-tenths of a gram of the preparation was injected into the buttocks in the usual manner. Ten days later the lesions had entirely disappeared, leaving only slightly pigmented areas where they had previously existed.

Case XII.—Castor; age 18 years; duration of disease about 1½ years. The lesions consist of granulomatous tumors measuring from 5 centimeters in diameter, situated anteriorly over the right ankle, over the right external malleolus, above the right knee, and posteriorly over the right ankle, over the left ankle, above the heel and above the left knee. They are usually covered by yellow crusts. Over the left elbow posteriorly and the right ankle there are old scars. A few spirochætæ were found in a smear from one of the granuloma. The patient was given 0.3 gram of the preparation in the usual manner. Fifteen days later the active lesions had entirely disappeared. I have not been able to see this patient since that date.

Case XIII.—(Plate IX.) Leon; age 12 years; duration of the disease a little over a year. No others in the family have yaws. The lesions are situated anteriorly over the forehead, neck, axillae, bends of the elbows, knees, legs, ankles, scrotum, penis and foreskin, posteriorly over the neck, shoulders, posterior surface of right arm and forearm, both elbows, back, left thigh, gluteal fold, posterior surface of legs and left heel. The lesions consist of granulomatous tumors varying in size from about 5 millimeters to 3 and 4 centimeters in diameter, usually covered by yellow crusts. On the lower legs a number of the granulomata are circular in outline, the skin in the center being depressed. Evidently ulcerations will occur in these central areas should the case remain untreated. There is an ulcer on the left heel measuring about 3 centimeters in diameter. On the scrotum, penis and foreskin there are 9 papillomata measuring about 4 to 5 millimeters in diameter. The granulomatous tumors are raised from 0.5 to 1.5 centimeters above the surface of the skin. For the character and distribution of the lesions Plate IX, figs. 1 and 2 may be consulted. Three tenths of a gram of the preparation was injected in the usual manner into the buttocks. Twenty days later the lesions had entirely disappeared, the skin was everywhere perfectly smooth and the ulcer on the heel entirely healed (see Plate IX, figs. 3 and 4). Since this time the patient has remained well.

Case XIV.—(Plate X.) Candido; age 10 years; duration of the disease 2 years. The lesions are situated anteriorly over the chin, left side of neck, below the left shoulder, at the bend of the left elbow, the right forearm, the left thigh, both knees, right leg and right ankle, and posteriorly over the neck, shoulders, back, buttocks and right leg. The lesions consist of patches, round or irregular in outline, sharply circumscribed, some as small as 3 millimeters, but generally 4 or 5 centimeters in diameter. They are usually covered with yellow crusts, although in some instances they show reddened summits. They are usually raised about 0.5 centimeter above the surrounding surface of the skin. There are many pigmented spots on the anterior surface of the thighs, which are evidently the result of healed lesions. (For the distribution and character of the lesions see Plate X, figs. 1 and 2.) The patient was given 0.3 gram of the preparation in the usual manner. When seen twenty days later, the lesions had entirely disappeared, the skin was everywhere entirely smooth, only pigmented patches remained where the lesions had previously existed (see Plate X, figs. 3 and 4). He since has remained well.

Case XV.—(Plate XI.) Nicomedes; age 8 years. The lesions are situated over the left eyebrow, chin, about the left shoulder, the arms, bends of the elbows, left forearm, knees, legs, left ankle, and posteriorly over the buttocks, gluteal fold, thighs and legs. There is a superficial ulcer measuring about 4 centimeters in diameter over the right heel. The lesions consist of granulomatous tumors measuring about 3 millimeters to 3 centimeters in diameter. They are sharply circumscribed, and raised about 4 or 5 millimeters above the surrounding skin. Many of them are covered with yellow crusts. Some of the smaller lesions appear as reddened papules, others as mushroom-like granulomatous masses. For their distribution and character see Plate XI, figs. 1 and 2. The patient was given 0.3 gram of the preparation in the usual manner. Twenty days afterwards the lesions had practically entirely disappeared (see Plate XI, figs. 3 and 4). He since has been well with the exception of having contracted a severe case of scabies, in some of the lesions of which *Sarcoptus scabei* was found. No urticarial eruption followed the injection.

Case XVI.—(Plate XII.) Felix; age 12 years; duration of the disease 8 years (?). The lesions are situated anteriorly over the chin, neck, left axilla, on the right arm, below the right axilla, the bends of the elbows, above the knees, the left foot, over the fourth and little toe, and posteriorly over the neck, shoulders, back, elbows, forearms, buttocks, above the popliteal spaces, legs and right ankle. The lesions consist of papillary granulomatous tumors varying from 4 millimeters to 3 centimeters in diameter, round or irregular in outline, and raised about 5 millimeters above the surrounding skin. They are usually covered with yellow crusts, or when not so covered they are red in color. Small blood vessels may be seen traversing these as a net work. Numerous pigmented spots are present over the knees, legs, ankles and buttocks, the result of older lesions. On the fourth toe of the left foot there is an ulcerating granulomatous lesion. For the distribution and character of the lesions see Plate XII, figs. 1 and 2. The patient was given 0.3 gram of the preparation in the usual manner. Twenty days later the lesions had entirely healed, the skin was everywhere smooth, only very slightly reddened areas of skin remained where they had previously existed (see Plate XII, figs. 3 and 4). The patient since has remained entirely well.

Case XVII.—Esteban; age 12 years; duration of the disease one year. The lesions consist of papillary granulomata situated anteriorly over the shoulders, bend of the left elbow, and posteriorly over the left elbow. They measure about 2 to 3 centimeters in diameter, and are raised above the surrounding tissue. Over the right heel is a partially ulcerated granulomatous lesion measuring about 3 centimeters in diameter. There are numerous scars over the chest, which are the result of old lesions. The patient was given 0.3 of a gram of the preparation in the usual manner. When seen twenty days after the injection the lesions had entirely disappeared, and the skin was everywhere entirely smooth. He since has remained well.

Case XVIII.—Juan; age 8 years; duration of disease 4 months. There is an ulcerating granuloma measuring about 3 centimeters in diameter situated over the anterior surface of the right leg. Over the left leg, posteriorly, there are five granulomatous lesions measuring 5 millimeters to 1 centimeter in diameter, covered by yellow crusts. There are numerous scars over both legs which are the result of earlier lesions. The patient was given 0.3 gram of the preparation in the usual manner. Twenty days later all signs of the lesions had disappeared, the skin was entirely smooth. He since has remained well.

Case XIX.—(Plate XIII.) Francisco; age 24 years; duration of the disease 7 months. He is unmarried, and has never had syphilis. No scar on the penis. The lesions are situated anteriorly over the neck, shoulders, chest, abdomen, arms, forearms, and thighs, and posteriorly over the neck, shoulders, back, arms, buttocks and legs. The lesions consist of numerous small pappillomata measuring from 2 to 4 millimeters in diameter, sharply circumscribed, and raised from 3 to 4 millimeters above the surrounding skin. Some of the larger ones are covered with yellow crusts. For distribution and character of the lesions see Plate XIII, figures 1 and 2. The patient was given 0.4 gram of the preparation in the usual manner. Fifteen days after the inoculation the skin was perfectly clear and smooth (see Plate XIII, figures 3 and 4). He since has remained well.

Case XX.—Tiburcio; age 10 years; duration of the disease is stated to be one month. Evidently yaws has been present for a longer time. The lesions are situated anteriorly over the right chest, below the clavicle, left breast below the nipple, both forearms, scrotum, left thigh, above the right knee, and posteriorly over the forearms, left buttock, both thighs, legs, and left ankle. The lesions consist of granulomatous tumors measuring usually about 1 to 3 centimeters in diameter, sharply circumscribed, round or irregular in outline, and raised for a distance of about 4 or 5 millimeters above the surrounding skin. Many of them are covered with yellow crusts; in the case of others they are red in color and the surface uneven. The patient was given 0.3 gram of the preparation in the usual manner. After twenty days the skin was entirely clear and smooth. He since has remained well.

Case XXI.—Maximo; age 5 years; duration of the disease 5½ months. The lesions are situated about the mouth, on the upper and lower lips, on the chin and neck, in the left axilla and on the posterior surface of the scrotum. They consist of granulomatous tumors measuring from 6 to 10 millimeters in diameter, and raised from 3 to 5 millimeters above the surrounding skin. The patient was given 0.25 gram of the preparation in the usual manner. After nine days the lesions had completely disappeared and the skin was perfectly smooth. He since has remained well.

Case XXII.—Ling (Chinaman); age 35 years. The lesions consist of five granulomata measuring from 1 to 1.5 centimeters in diameter, situated on the left leg and above the left knee. There is also an ulcerating granuloma measuring about 2 centimeters in diameter on the left ankle. A smear from one of these granulomata shows numerous spirochætæ. The patient was given 0.45 gram of the preparation in the buttocks. After three weeks the granulomatous lesions had entirely disappeared and the ulcer entirely healed. He since has remained well.

Case XXIII.—Pedro; age 18 years; duration of the disease 4 months. The lesions are situated anteriorly over the forehead, cheeks and chin, the chest, shoulders, arms, and forearms, thighs and legs; posteriorly over the shoulders, arms, chest, and legs. They consist of papules measuring from about 3 to 6 millimeters in diameter and raised for a distance of 3 to 4 millimeters above the surrounding skin. A few are covered with yellow crusts. The patient was given an injection of 0.4 gram of the preparation. After 18 days the skin was perfectly smooth, and only dark pigmented spots remained where the papules had formerly existed. He since has remained well.

Case XXIV.—Pablo; age 14 years; duration of the disease 2½ years. The patient has been treated at St. Luke's Dispensary for 1½ years. The condition was formerly diagnosed "fungus foot." At St. Luke's Dispensary he has treated with mercury and iodides internally, and locally with mercurial ointment, bichloride of mercury, lysol, Peru balsam, silver nitrate and permanganate of potash dressings. The lesions were also cauterized and curetted on several occasions. Very slight improvement followed all of this treatment. When seen by the author the lesions were confined to the right foot and ankle. Above the external malleolus there were four ulcerating granulomata measuring from 1 to 1.5 centimeter in diameter. Over the dorsum of the foot there was a large ulcerating granulomatous mass measuring about 6 centimeters in diameter. The greater part of the sole of the foot was also occupied by a larger ulcerating mass, the margins of which were very irregular in outline. Spirochætæ were found in several smears from the ulcerating granulomata. A diagnosis of tertiary yaws was made, and the patient was injected with 0.35 gram of the preparation in the usual manner. Before this treatment he had complained of much pain in the foot. Six days after the injection he stated that the pain had entirely disappeared. He was seen occasionally by his physician during the next two weeks who reported that a very great improvement had taken place in the condition of the foot, and that the lesions were rapidly healing. He was not seen by the author until 1½ months after the injection. At this time the lesions which existed formerly had entirely disappeared. Where the ulcer and granulomata formerly had been present, only firmly contracted scar tissue remained. Above the external maleolus patches of pigmented skin were present. The patient since has remained entirely well.

Case XXV.—(Plate XIV.) Juan, age 16. Duration of the diseases 3 weeks, during which time he has been treated with Chinese medicine containing iodides but with no favorable result. Denies syphilis. No sore or scar on penis. The lesions are situated on the forehead, cheeks, nose, upper lip, chin, neck, chest, abdomen, scrotum, shoulders, arms, forearms, palms of hands, thighs, knees, legs, ankles and feet; and posteriorly over the neck, shoulders, back, arms, elbows, forearms, buttocks, legs and ankles. The distribution is almost universal, although the lesions are more marked on the face and upper portion of the trunk. They consist of papules measuring from about 3 millimeters to one centimeter in diameter; the largest are situated on the face. They are sharply circumscribed, the largest ones raised for a distance of 3 to 4, the smallest for a distance of 1 to 2 millimeters above the surface of the surrounding skin. On the face and on many parts of the chest the papillary tumors are covered with yellow crusts. Elsewhere the papules are slightly reddened, with dry surfaces. Smears from the papules show numerous spirochætæ. For the character and distribution of the lesions see Plate XIV.

The patient was given an injection of 0.38 gram of the preparation in the buttocks in the usual way. The spirochætæ had disappeared from the lesions three days later. The lesions gradually disappeared; after twenty days the skin was clear, as may be seen by Plate XIV. The patient since has remained well.

CONCLUSION.

Dioxy-diamido-arsenobenzol appears to be an ideal specific for yaws. Three or four days after the injection of the drug, the granulomatous lesions begin to disappear and in the course of from ten to twenty days they usually have disappeared entirely leaving a perfectly smooth, pigmented skin where the lesions previously existed. The absorption of tumor masses measuring several centimeters in diameter and about a centimeter in thickness in so short a time, and under the influence of no local treatment whatever, has been very striking and surprising. Indeed, in the severe cases the disappearance of the lesions and the cures produced could be most aptly spoken of as marvelous. Even in cases where large granulomatous masses or deep ulcerations existed, these were healed within from two to four weeks. I do not know of any more striking example in medicine of a specific action than of that produced by dioxy-diamido-arsenobenzol on the lesions of yaws.

A slight reddening appears about the margins of the lesions in from twenty-four to forty-eight hours after the injection of the preparation. The center of the lesion then usually assumes a purplish or bluish, congested appearance. Phagocytes are attracted to this area which now assumes a grayish or brownish hue. The spirochætæ quickly disappear and the granulomatous tissue becomes absorbed, leaving dark, pigmented areas of skin which later resume their normal color. In the cases where crusts have existed, these frequently are not absorbed, but drop off.

As stated above, none of the cases have shown any signs of relapse, although they received but a single injection, and over four months have elapsed since most of them were inoculated.

Nevertheless, I believe that in a few of the most severe instances of the disease, where the ulcerations are extensive and of long standing, a second inoculation given about three weeks after the first one may be advisable. In a case of syphilis in a European who had been treated with mercury and iodides, according to his statements, for two years, in which the testicle was swollen to a diameter of about 15 centimeters and a large ulcer measuring about 10 centimeters in diameter was situated on the scrotum, 0.4 gram of dioxy-diamido-arsenobenzol was given by injection into the buttocks. A very rapid diminution in the size of the testicle and of the ulcer occurred during the following three weeks, but the ulcer did not entirely heal nor did the testicle reach its normal size until a second intravenous injection of 0.4 gram of the drug was given. Then apparently a perfectly normal condition was brought about. Only in Number IV of the yaws cases which I treated with this preparation was there any question as to whether a second dose should have been given and as to whether a second one might not have hastened the cure.

However, the lesions eventually entirely healed as a result of the primary injection. The dose of the preparation for the treatment of yaws should be from 0.25 to 0.3 gram for children and from 0.4 to 0.5 gram for adults. Smaller amounts may not give such favorable results.

Some observers believe that it is not advisable to attempt to repress the eruption of yaws, as this may be accompanied by considerable constitutional disturbance. The employment of dioxy-diamido-arsenobenzol simply caused the eruption to fade away. There were no unfavorable constitutional symptoms other than an occasional transitory rise of temperature produced thereby, and the patients seemed improved in health following the disappearance of the eruption.

A number of authors have also laid great stress upon the local treatment of the disease. If dioxy-diamido-arsenobenzol is employed, the local treatment is entirely unnecessary, except in cases of severe ulceration and where organisms other than *Treponema pertenuis* have invaded the lesions. Case IV would undoubtedly have been cured more quickly if the ulcerating granulomatous masses had been curetted, disinfected, and dressed daily with sublimate solutions, since spirochætæ other than *Spirochaeta pertenuis* and bacilli and cocci were present in the lesions. However, even in this case they eventually entirely healed without local treatment.

The cure of yaws by this preparation, and the successful results which have been obtained with it in the treatment of certain cases of syphilis, may suggest again the identity of yaws and syphilis. It does not appear strange that this preparation should have such a destructive action against both *Treponema pertenuis* and *Treponema pallidum,* since both from a morphologic and biologic standpoint these two organisms appear closely related. Indeed, potassium iodide, which is so valuable in the treatment of syphilis, has hitherto been the most successful remedy employed in the treatment of yaws. As has been pointed out by other observers, the therapeutic argument for the identity of yaws and syphilis and hence for the identity of their specific organisms, is very fallacious. Many instances in medicine might be quoted in support of this statement. Quinine which destroys certain forms of *Plasmodium malariæ, Plasmodium vivax,* and *Laverania malariæ,* also destroys *Amœba dysenteriæ.*

In conclusion it would appear that dioxy-diamido-arsenobenzol is as important a specific for yaws as quinine is for malaria. Therefore, a fourth medicinal specific in medicine has been discovered.

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ILLUSTRATIONS.

PLATE I. Case I before treatment.

II. Case I twelve days after treatment. The skin is everywhere perfectly smooth. In the photograph of the posterior view it appears that tumors still exist in the region of the popliteal spaces. This appearance is due to the areas of pigmented skin. The granulomata here as elsewhere have entirely disappeared and the skin is entirely smooth.

III. Case II before treatment.

IV. Case II nine days after treatment. The skin is everywhere smooth. Pigmented patches are visible on the lower extremities both anteriorly and posteriorly.

V. Case III before treatment.

VI. Case III fourteen days after treatment.

VII. Case IV before treatment.

VIII. Case IV after treatment.

Fig. 1. Nine days after treatment. The lesion on the chin is not entirely healed. At the bends of the elbows the character of the lesions has entirely changed. The formation of scar tissue is evident.

Figs. 2 and 3. Three weeks after treatment. There are scabs over the bend of the left elbow anteriorly, and posteriorly over the right elbow and popliteal spaces. These have since disappeared and the skin has become smooth.

IX. Case XIII.

Figs. 1 and 2. Before treatment.

Figs. 3 and 4. Twenty days after treatment. The skin is entirely smooth.

X. Case XIV.

Figs. 1 and 2. Before treatment.

Figs. 3 and 4. Twenty days after treatment.

XI. Case XV.

Figs. 1 and 2. Before treatment.

Figs. 3 and 4. Twenty days after treatment.

XII. Case XVI.

Figs. 1 and 2. Before treatment.

Figs. 3 and 4. Twenty days after treatment.

XIII. Case XIX.

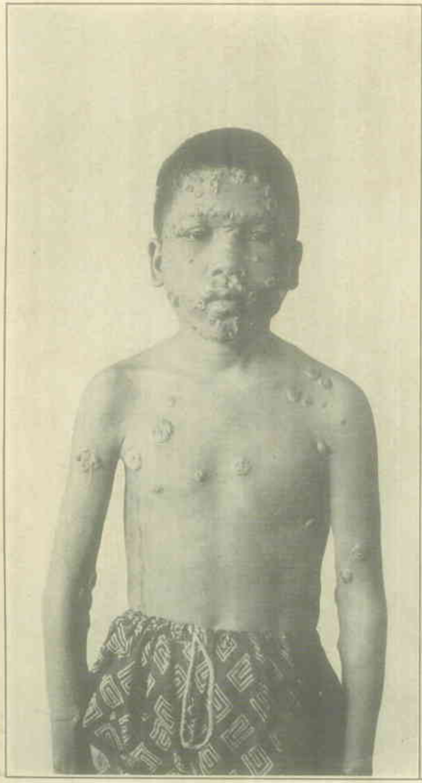
Figs. 1 and 2. Before treatment.

Figs. 3 and 4. Twenty days after treatment.

XIV. Case XXV.

Figs. 1 and 2. Before treatment.

Figs. 3 and 4. Twenty days after treatment.



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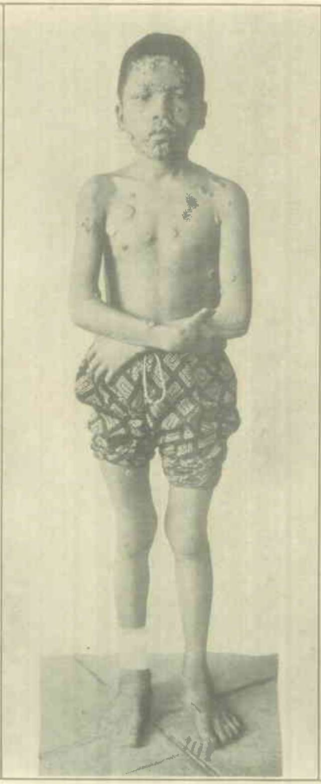

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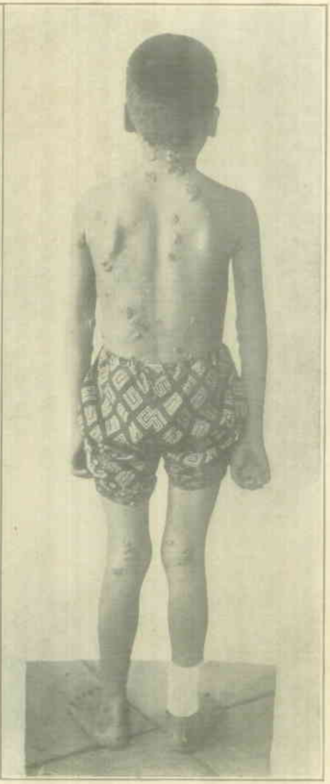
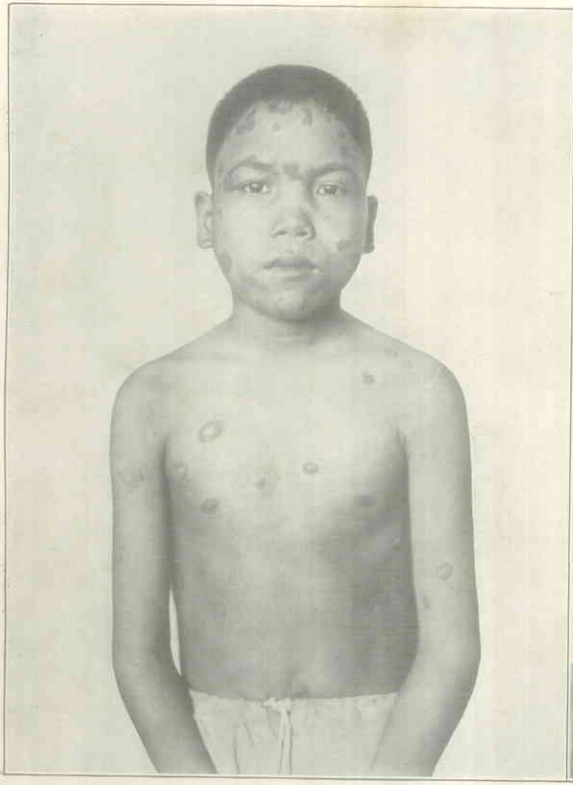


FIG. 3.

PLATE I.



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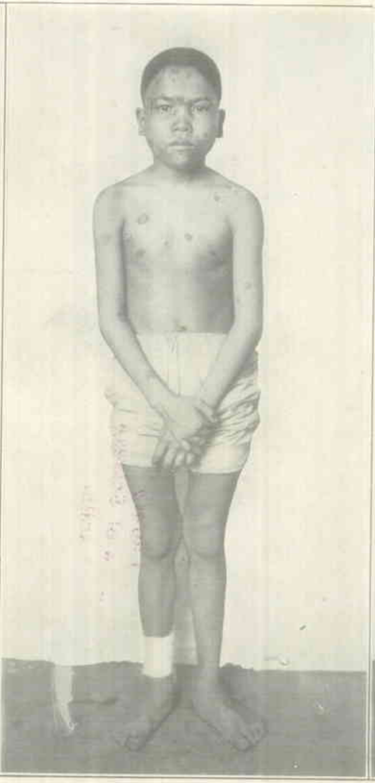

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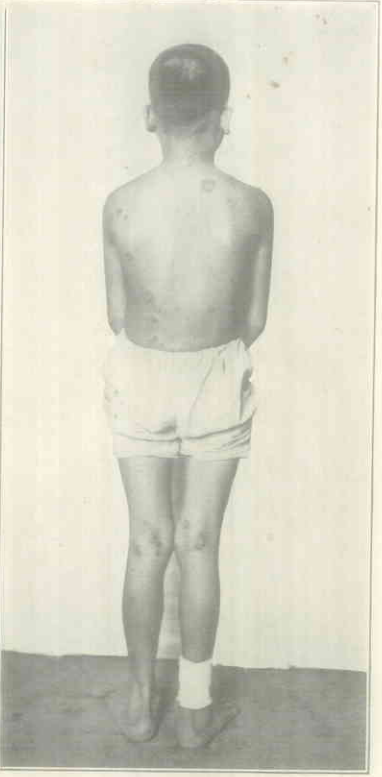

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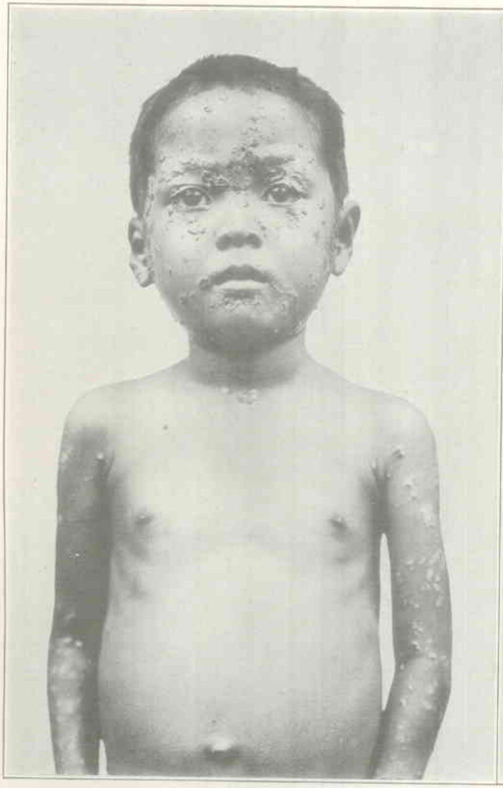

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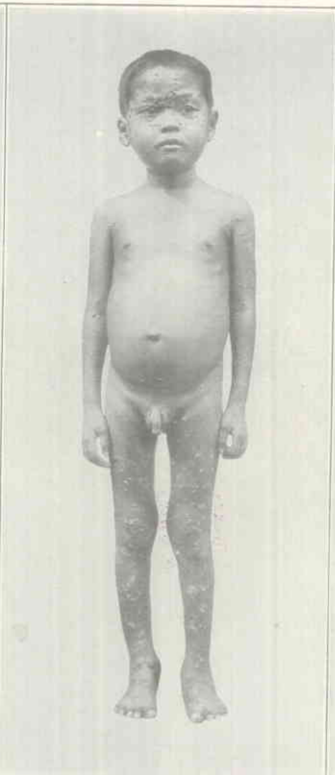

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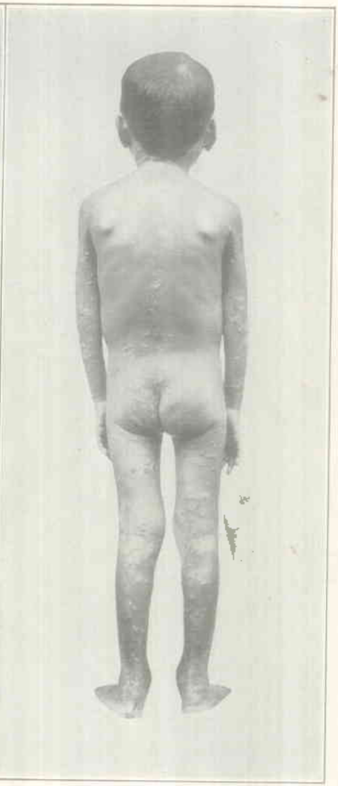

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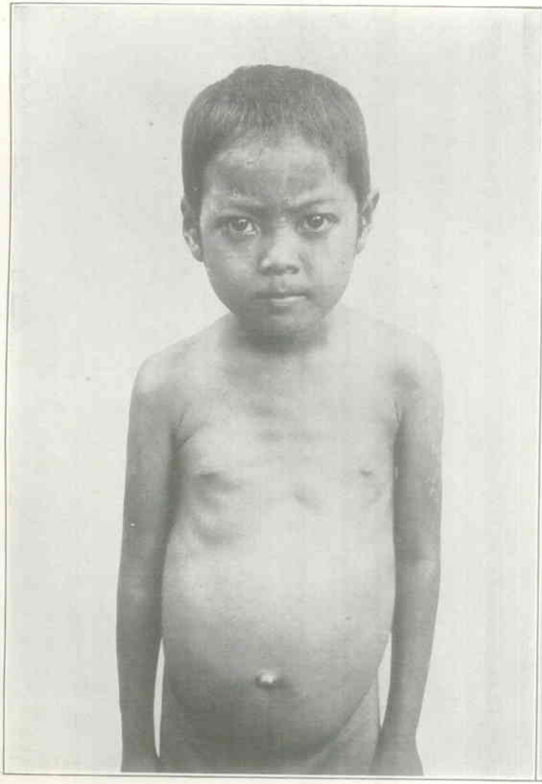

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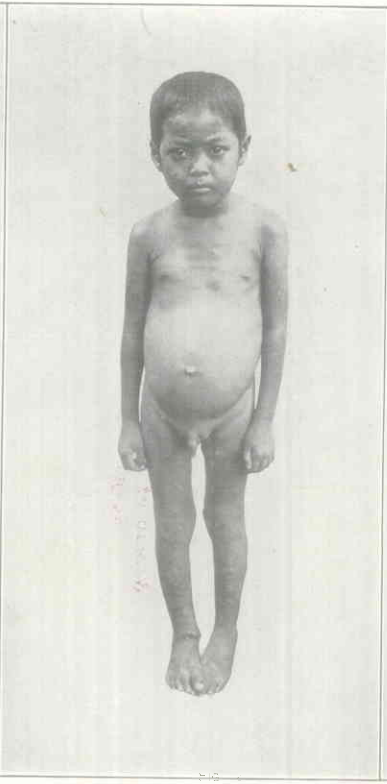

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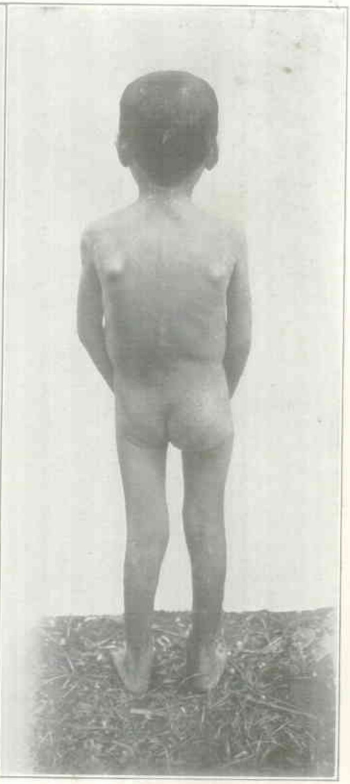

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PLATE IV.

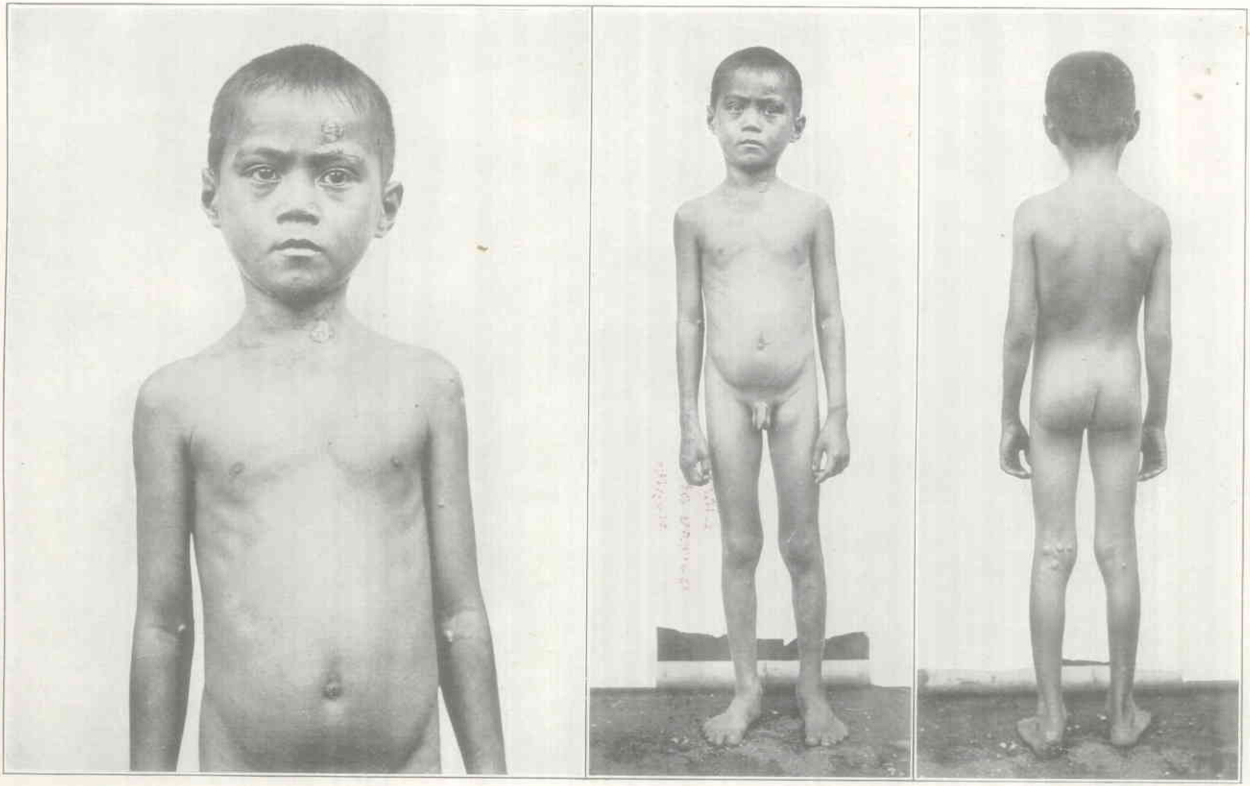

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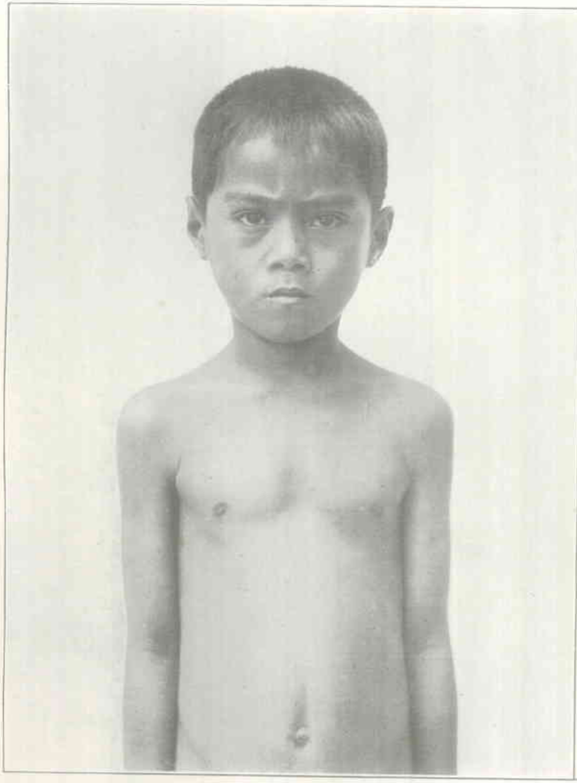
PLATE V.



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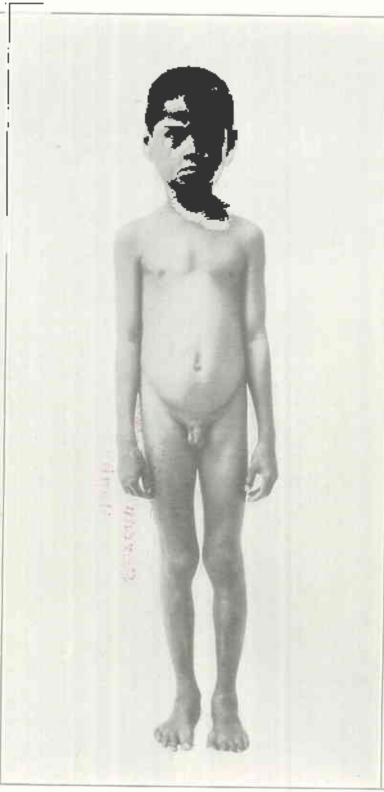

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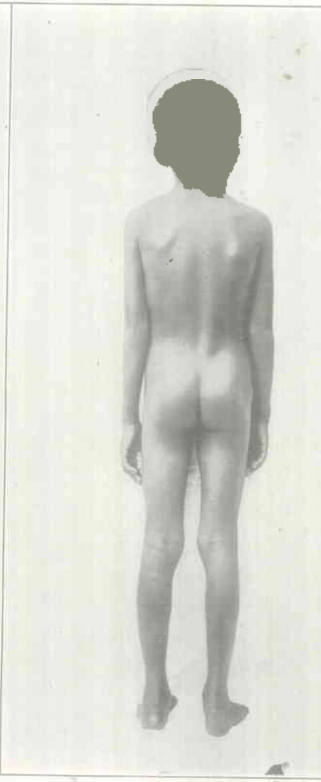

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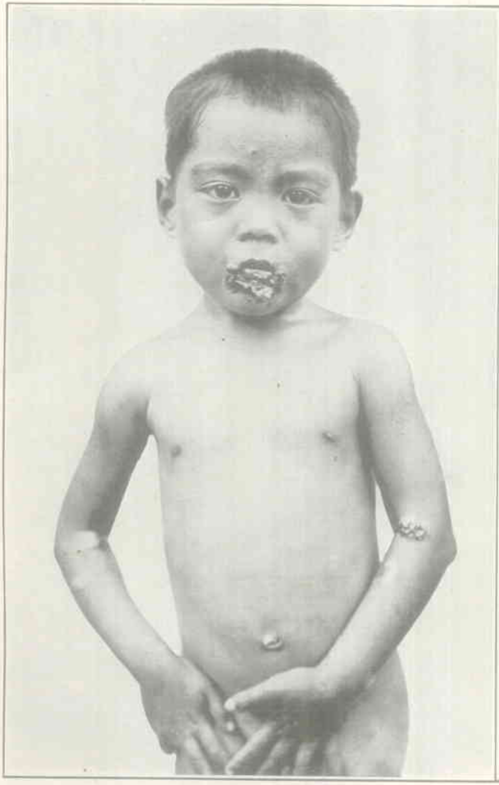

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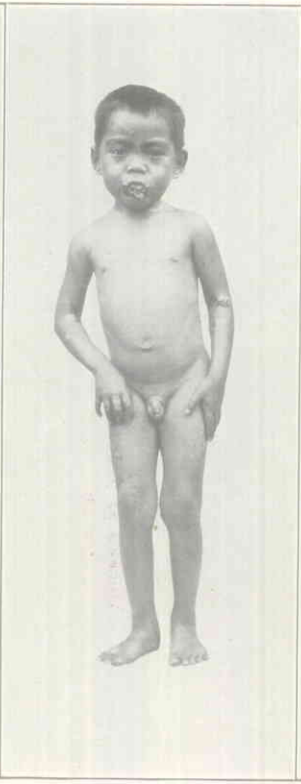

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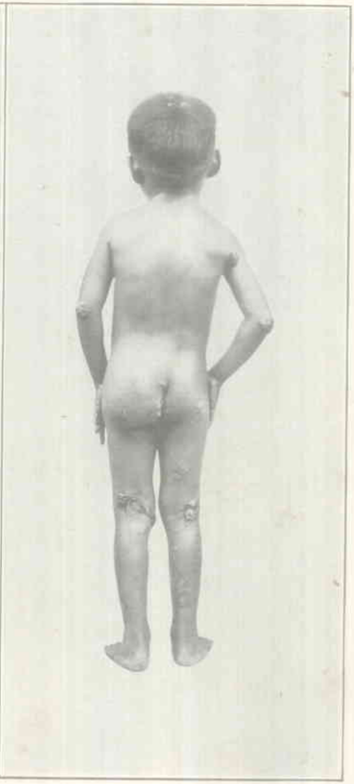

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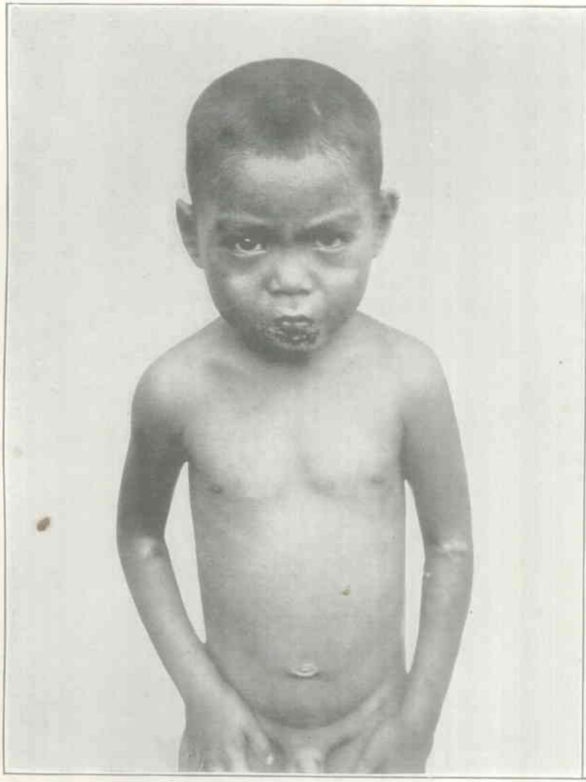

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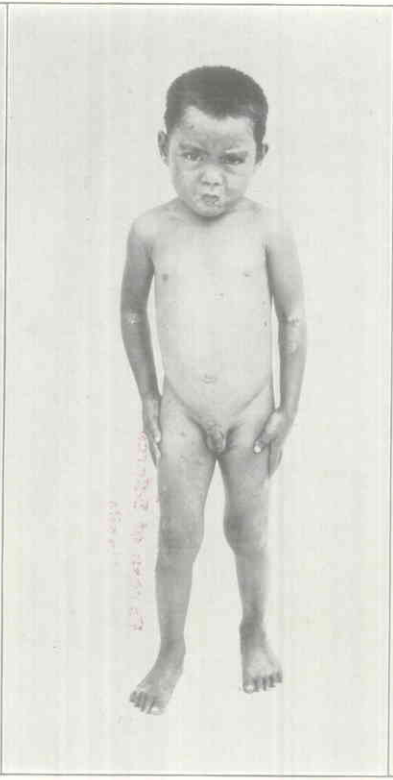

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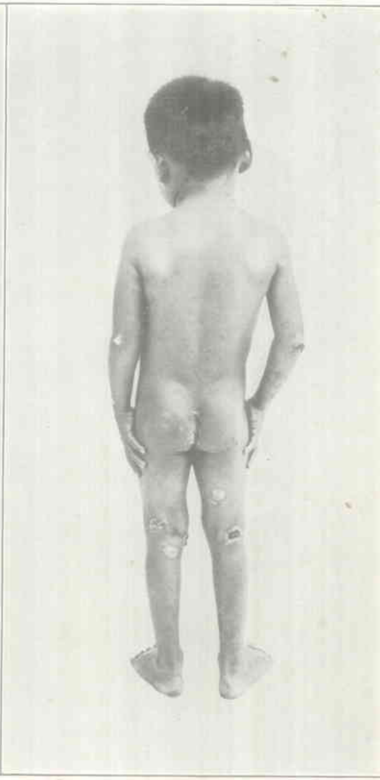

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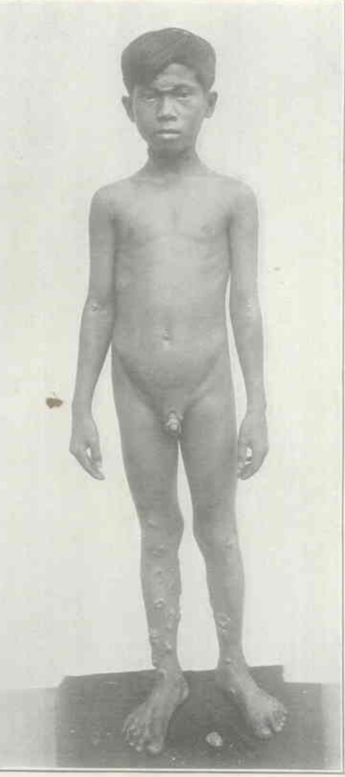
PLATE VIII.



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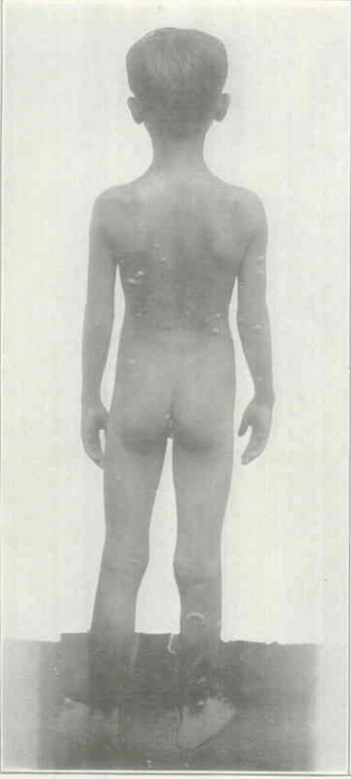

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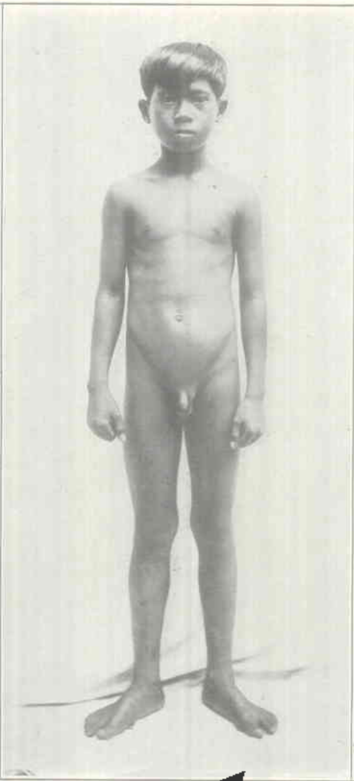

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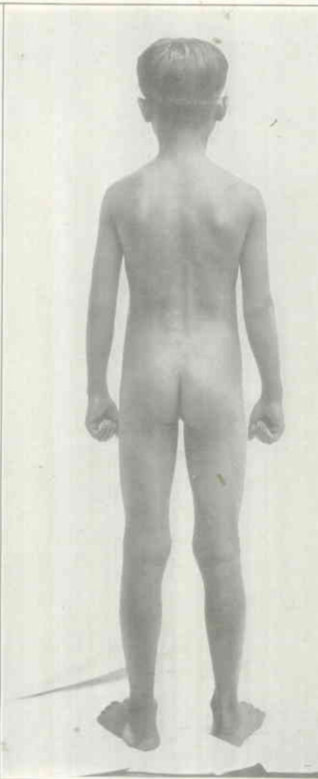

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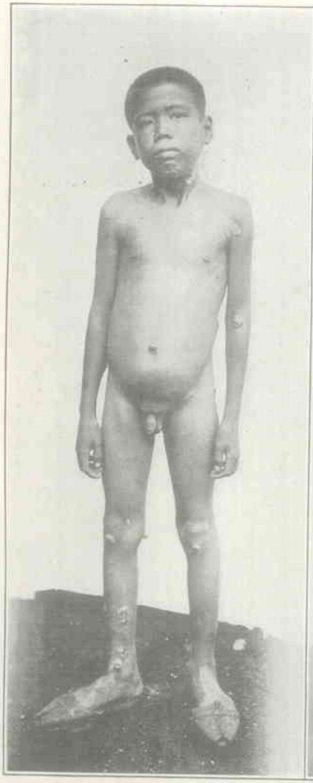
PLATE IX.



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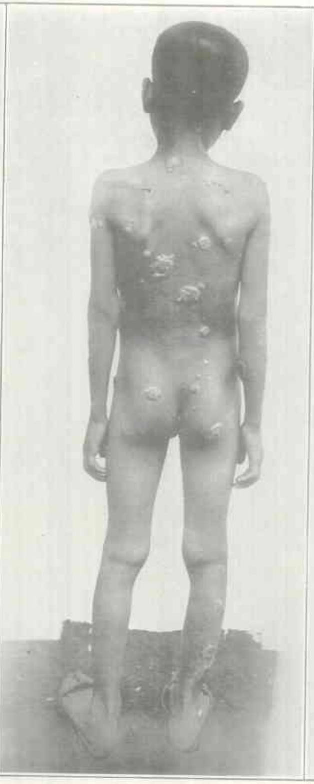

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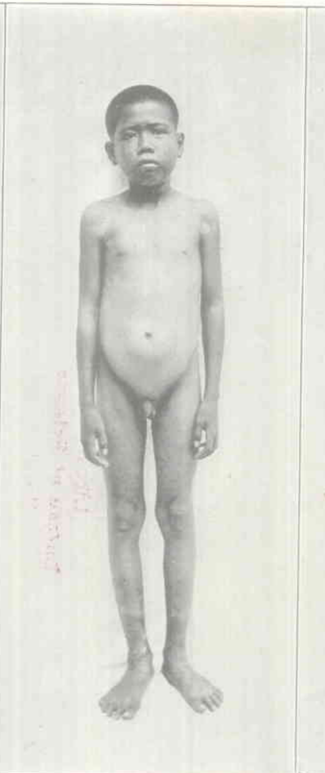

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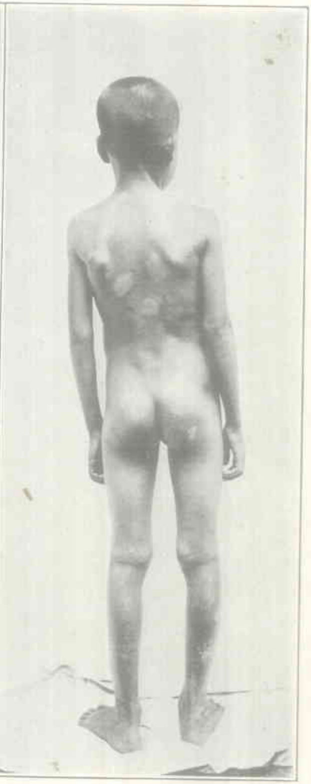

FIG. 4.

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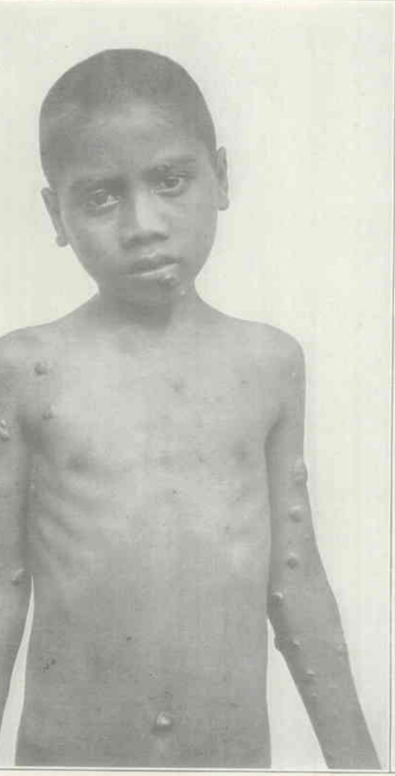

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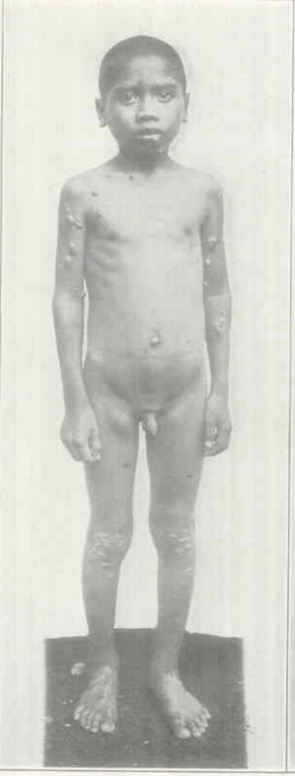

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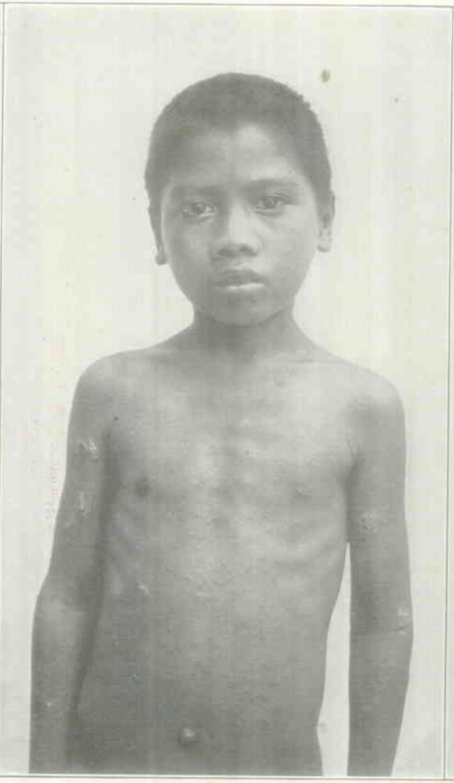

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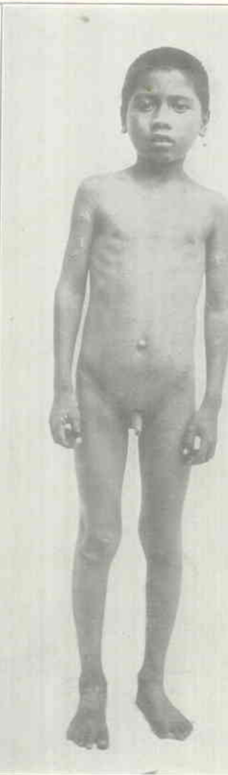

Fig. 4.

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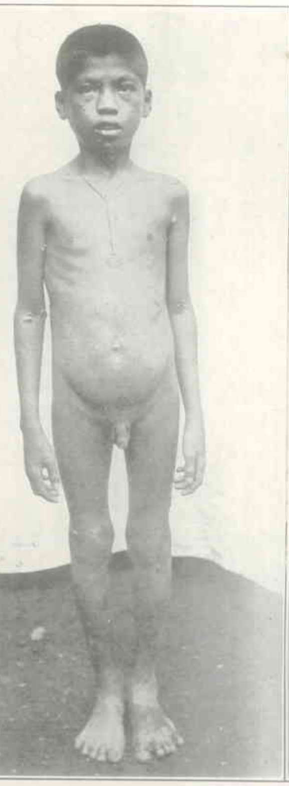

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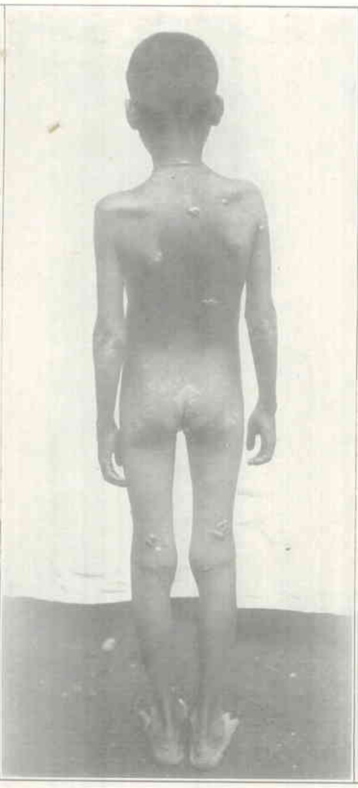

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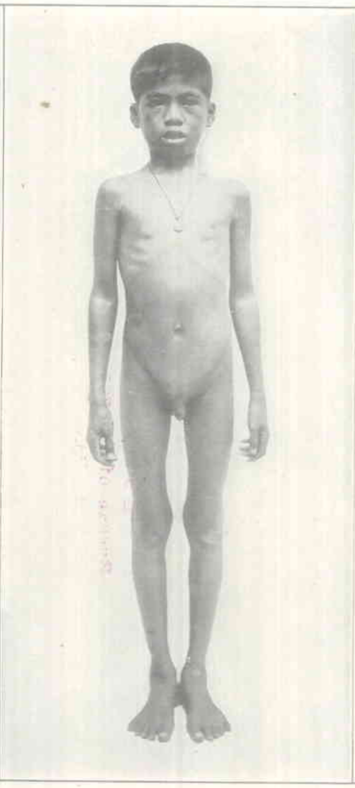

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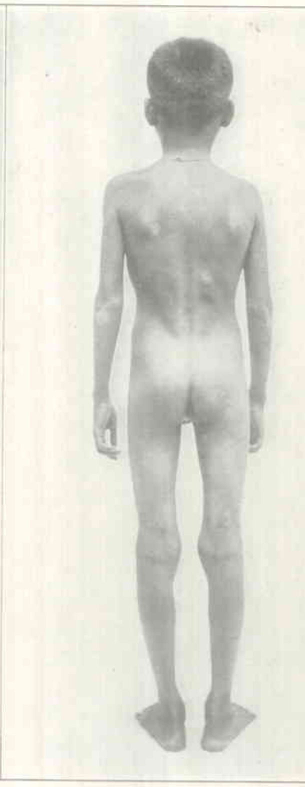

FIG. 4.

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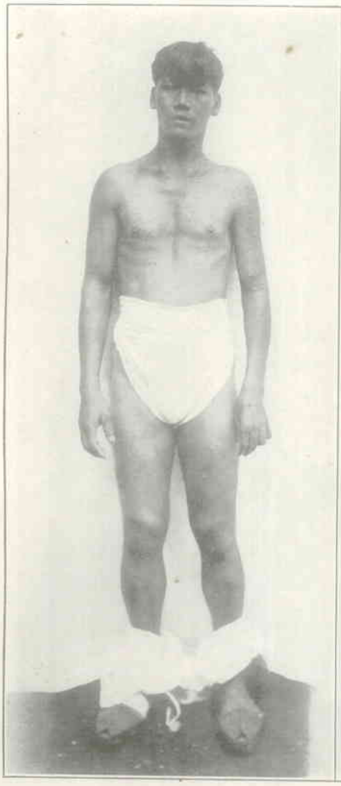

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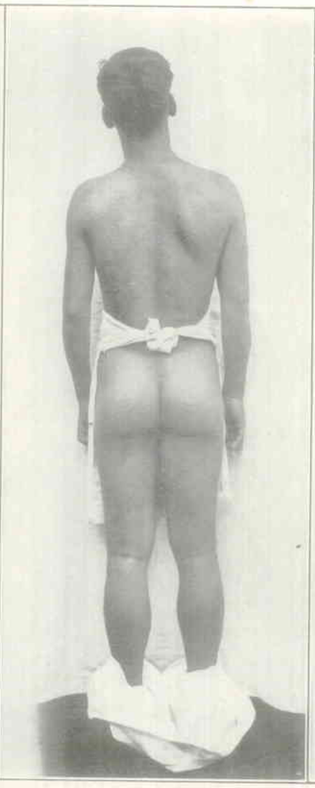

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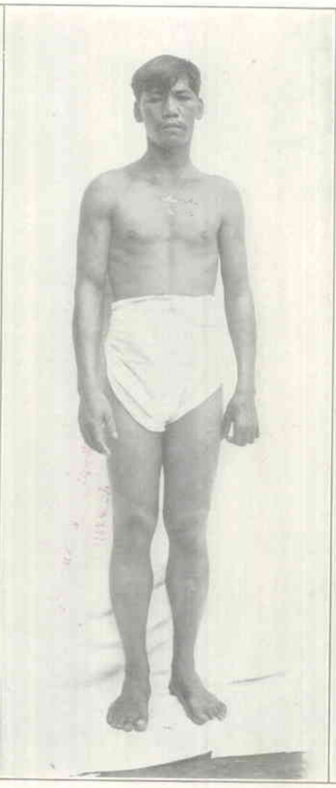

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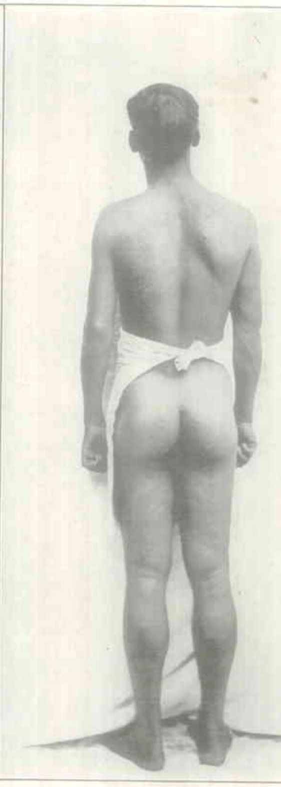
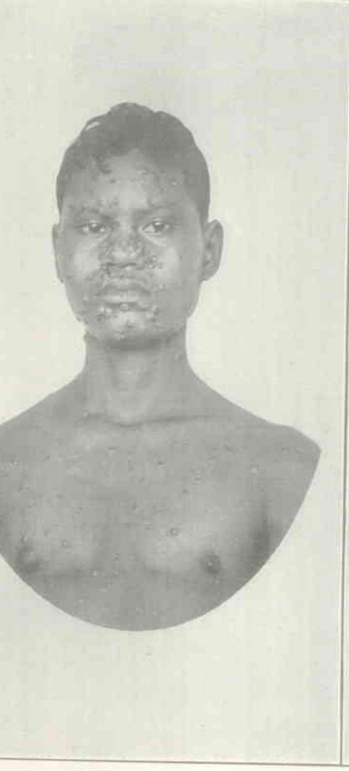


FIG. 4.

PLATE XIII.



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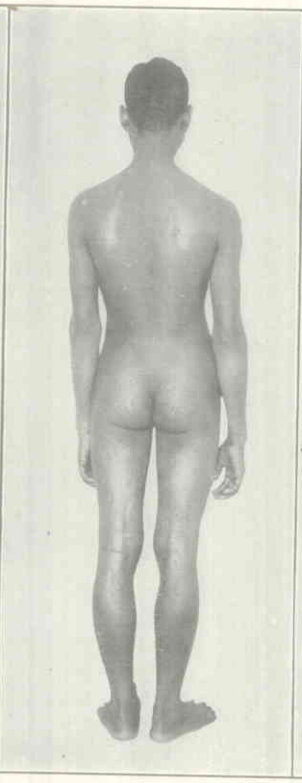

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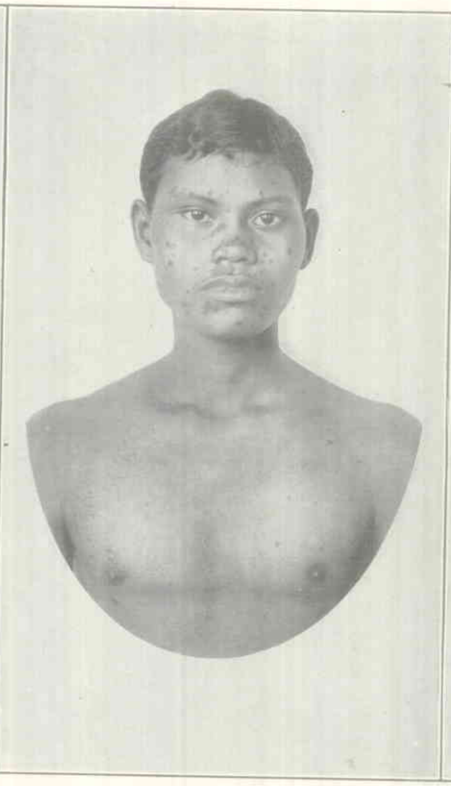

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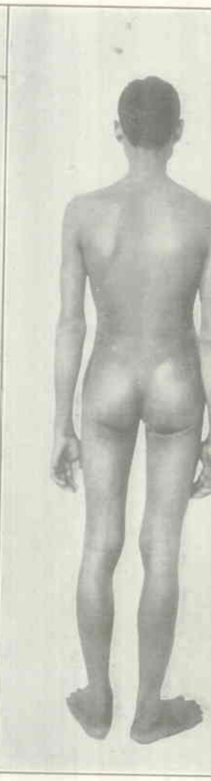

FIG. 4.

PLATE XIV.

REVIEWS.

A Textbook of Experimental Physiology For Students of Medicine. By N. H. Alcock, M. D., D. Sc., and F. O'B. Ellison, M. D. Cloth. Pp. xii+139, 36 illustrations. Price $1.50 net. Philadelphia: P. Blakiston's Sons, 1909.

This book, with a preface by Ernest H. Starling, gives a clear description of the experiments which should be performed by medical students in the physiological laboratory. Not only are the usual muscle and nerve experiments included, but also the more important ones on mammals. The idea of the authors is not simply to show the student *how to experiment,* but rather to teach him *how to observe.* The physiologic anatomy of the rabbit is an especially excellent example of this method of instruction. Some experiments, such as the demonstration of the action of secretin on pancreatic secretion, or the analysis of the gases of the blood by Haldane's apparatus, seem to the reviewer to be somewhat difficult for an elementary course. Certain procedures are inserted to comply with the restrictions which are placed by law upon vivisection in Great Britian.

HANS ARON.

Examination of the Urine. A Manual For Students and Practitioners. By G. A. DeSantos Saxe, M. D. 2d ed. Cloth. Pp. 448. Price $1.75. With text illustrations and colored plates. Philadelphia and London: W. B. Saunders Company, 1909.

Modern clinical medicine demands a certain amount of laboratory work from nearly all practitioners and this book is destined as a guide for such clinical examination of urines. The first part, "Chemic Examination of the Urine," is hardly more than a careful compilation of what is found in a great number of other text and hand books. The second part, "Microscopic Examination," especially the chapters on "Urinary Diagnosis" and "Functional Renal Diagnosis," probably never has been treated so clearly and thoroughly. The author gives here a number of original pictures and many practical suggestions based on his own experience. The clinical significance and importance of the different findings are well discussed, making the book of real value for the practicing clinician.

The questions at the end of each chapter remind one very forcibly of the so-called *vade mecum* used by students not of the best type just before examination, and impress the reviewer as somewhat out of place in a work of this character.

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